

**UNIVERSITY OF CAPE COAST**

**EFFECT OF TEMPERATURE ON POPULATION DYNAMICS OF  
*MELOIDOGYNE* SPP. AND *FUSARIUM* SPP. INFESTING  
SWEET PEPPER IN NIGER**

**BOUBACAR TOUKAL ASSOUMANA**

**2016**

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University of Cape Coast

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SWEET PEPPER IN NIGER**

BY

**BOUBACAR TOUKAL ASSOUMANA**

Thesis submitted to the Crop Sciences Department of the Agricultural School,  
University of Cape Coast, in partial fulfilment of the requirements for the  
award of Doctor of Philosophy degree in Crop Science

JUNE 2016

## DECLARATION

### Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

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### Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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## ABSTRACT

*Meloidogyne* spp. and *Fusarium* spp. cause wilt disease in sweet pepper and result in decline of its production. Other limiting factors of sweet pepper production are inappropriate agronomic practices and lack of effective climate adaptation strategies. The aim of this study was to investigate on one hand, how farmers perceive climate change and their access to agricultural extension services and, on the other hand the distribution and identification of causal agents of wilt disease and also to find out the effect of temperature on the co-infection of pathogens on sweet pepper. The results revealed that the perception of farmers on rainfall patterns do not match with past meteorological records. Respondents indicated that there is an increase in temperature which is in agreement with climatic data evidence. Crop diversification was found as the major adaptation strategy to climate variability. The lack of sufficient access to information is the constraints that hindered farmers to adapt effectively. The incidence of the *Meloidogyne* spp. was 100% in both districts while the occurrence of *Fusarium* spp. was 80.32% and 62.23% in Diffa and Aguié respectively. Based on morphological and molecular characterization, three pathogens were identified in sweet pepper; *Meloidogyne enterolobii*, *Meloidogyne incognita* and *Fusarium solani*. The sequential and concomitant inoculation of the fungus and/ or the nematode did not cause the sudden death of sweet pepper plants as observed in the field. It only resulted in significant increase in yellowing in leaves and high nematode and fungal reproduction in inoculated plants in comparison to the uninoculated plants. Temperature greatly influenced the reproduction capacity of *M. enterolobii* and *F. solani*.

## KEY WORDS

Effect of temperature

*Fusarium* spp.

*Meloidogyne* spp.

Niger

Perception of farmers

Sweet pepper

## ACKNOWLEDGEMENTS

I would like first to thank the German Federal Ministry of Education and Research for providing my doctoral scholarship through the West African Science Service Centre on Climate Change and Adapted Land Use (WASCAL). I am also grateful to Prof. Amoro (IPR/IFRA) and Dr. Nyarko (UCC).

I am again grateful to my Principal supervisor Dr. Mbaye Ndiaye, regional centre AGRHYMET for his guidance and help to enable me expand my knowledge in Plant Pathology. I would like to express my sincere gratitude to my co-supervisor, Dr. Grace van der Puije, University of Cape Coast, Ghana with whom I worked actively by distance despite her busy schedule. Her good humour and willingness to discuss new ideas have made working with her a unique and unforgettable experience.

I would like to thank my co-supervisor Prof. Dr Florian Grundler, INRES, University of Bonn for giving me the opportunity to work with him in his lab and with his team. I am deeply grateful to Dr. Naser, Dr Alexandre, Samer Habsah, PhD student, INRES, who helped with the identification of pathogens. I would like to thank to Mr. Etienne Sarr for providing me a good training in nematology, Prof. Sikora Richard, Prof. Sanoussi Atta and Dr. Haougui for their useful inputs. Many thanks to Dr. Dahiratou Ibrahim, Ecole Normale Superieure de Niamey and Dr Karmadine, Université de Niamey.

I am grateful to the regional centre AGRHYMET for supporting my field study and for providing meteorological data. Thanks are due my family for their encouragement of my academic pursuits. Special thanks to my fiancé, Mariama. Many thanks also to all my friends.

## **DEDICATION**

This thesis is dedicated to my family and in memory of my father. To my fiancé,  
Mariama.



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## LIST OF ACRONYMS

AGRHYMET	Centre for Agronomy Hydrology and Meteorology
bp	base pair
CLA	Carnation Leaf-pieces Agar
cm	centimetre
FSSC	<i>Fusarium solani</i> Species Complex (FSSC)
GDP	Gross domestic product
ha	hectare
INRES	Institute of Crop Science and Resource Conservation
IPCC	Inter-governmental Panel on Climate Change
J2	Second-stage juveniles
Kb	Kilo base
M.A	Ministry of agriculture
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar
RFLP	Restriction Fragment Length Polymorphism
RGAC	Recensement Général de l'Agriculture et du Cheptel
SSA	Sub-Saharan Africa
WASCAL	West African Science Service Centre on Climate Change and Adapted Land-use

## CHAPTER ONE

### INTRODUCTION

According to the Intergovernmental Panel on Climate Change IPCC (1997), Africa is the most vulnerable region to climate change because widespread poverty and limits adaptive capacity. The impacts of climate change on agriculture could seriously worsen livelihood conditions for the rural poor and increase food insecurity in the region. One strategy undertaken to improve sustainable food security and reduce poverty was the development of irrigation development of vegetables crops through irrigation schemes. One of the main problems that affect the yield of many crops is the biotic- and abiotic-stress. Nematodes (*Meloidogyne* spp.) and fungus (*Fusarium* spp.) infections, as one of the biotic stresses, cause considerable economical losses every year around the world. These losses are occurring on several economically important vegetables such as sweet pepper in Niger. It is important to identify the pathogens of economic importance in sweet pepper for designing effective management.

#### **Background to the Study**

The rural economy of Sub-Saharan Africa (SSA) remains strongly based on agriculture employing about 62% of the population and generating 27% of the GDP of these countries in 2005 (Staatz & Dembele, 2007). According to Stocker, Qin, Plattner, Tignor, Allen, Boschung and Midgley (2013), rising temperatures and changing precipitation patterns will likely lead to an acute decline in rainfed crop production in some of African countries. It

is estimated that, given current warming trends in SSA, the production of major cereals could decline by as large as 20% by mid-century (Schlenker & Lobell, 2010). For instance, previous studies on climate change impacts indicate that staple crops like maize (-5 %), sorghum (-14.5 %) and millet (-9.6 %) yields are set to decline significantly during the 21<sup>st</sup> century (Knox, Hess, Daccache, & Wheeler, 2012). This is a worsening situation considering that most smallholder farmers rely on rainfed agriculture in SSA. Unless measures are undertaken to mitigate the effects of climate change, food security in SSA will be a critical issue and under threat.

One of the alternatives undertaken to overcome and reverse the tendency of the production decline, is the development of irrigation and improvement in agricultural productivity. These variables are key, not only for future economic development, poverty reduction, and food security in SSA, but also for climate change adaptation (Calzadilla, Zhu, Rehdanz, Tol, & Ringler, 2009). Amongst irrigated crops, vegetables are of significant importance for smallholder farmers. According to Pena and Hughes (2007), vegetables are the best sources for overcoming micronutrient deficiencies and provide smallholder farmers with much higher income and more jobs per hectare than staple crops. Consequently, the worldwide production of vegetables has doubled over the past quarter century and the value of global trade in vegetables now exceeds that of cereals (Pena & Hughes, 2007).

Despite the importance of vegetables in ensuring food security under changing climate, little attention is paid on the pests and diseases responsible for heavy yield losses on crops. In fact combined infestation of pests and



diseases in plants could result up to 50% loss in attainable yield of major crops (Gautam, Bhardwaj, & Rohitashw, 2013). Plant diseases are estimated to cause yield reduction of almost 20% in the principal food and cash crops world-wide. For example in SSA, yield losses due to diseases in pepper production can reach up to 50% (Chadha, Oluoch, Saka, Mtukuso, & Daudi, 2003). Combination of climate variabilities and effect of diseases on crops could seriously worsen the livelihood of the rural poor and increase food insecurity in SSA.

Niger is one of such SSA countries most vulnerable to climate change and variabilities. Indeed a recent study on mapping of vulnerability and poverty in Africa listed Niger as one of the countries that are both most vulnerable to climate change and with the least capacity to respond (Orindi, Ochieng, Otiende, Bhadwal, Anantram, & Nair, 2006; Stige, Stave, & Chan, 2006). Agriculture is the main source of livelihood for most rural communities in Niger accounting for about 38.2% to GDP (World Bank, 2012). The rural sector employs over 80% of the working population, and it is also in rural areas that the highest numbers of poor people (86%) are found (Mahama & Boulenger, 2003). Agro-forestry-pastoral productions are the main resources of the country, and are very dependent on climatic conditions which are characterized by high spatial and temporal variability.

In Niger, climate constraints are one of the main factors limiting economic development. Since 1968 there has been recurrent drought with rainfall deficits. The water requirements for maximum yields of staple crops (pearl millet, sorghum and cowpea varieties) have not been satisfied in most

years and that resulted in rather recurring deficits of production sometimes generating food insecurity which exacerbates poverty. To meet the growing demand for food, the Niger government has elaborated strategic plans based on sustainable agriculture. Some of these alternatives include the reduction of pest outbreaks estimated presently at 30% of annual yield of production and the development of irrigated cropping. As a result, the area under vegetables cropping increased from 73 345 ha in 2006/2007 (RGCA, 2007) to 106, 801.59 ha in 2012/2013 (M. A, 2013). Amongst the vegetable crops, sweet pepper (*Capsicum annum* L.) is one of the high cash crops in Niger, particularly in Diffa and Aguié ensuring self-sufficiency and generation of income for farmers. It is currently the third exported crop after cowpea and onion and the monetary value of production was estimated at 18.05 million Euro with an average yield of 17 ton / ha in 2003 (Djibey, 2012). This yield obtained on the fields of farmers remains very low compared with the potential yield of 30ton/ha (Grubben & El Tahir, 2004).

The most important causes of this low yield are inappropriate agronomic practices, pests and diseases (Haougui, 1999; Haougui & Bizo, 2009). Among the various pests and diseases, the nematode-fungus disease complex particularly of *Meloidogyne* spp. and *Fusarium* spp. is the most important. Root Knot Nematodes (RKNs) (*Meloidogyne* spp.) allegedly cause damage and are of the most of economic importance (Haougui, 1999; Haougui & Bizo, 2009). The average yield losses due to RKNs are estimated to be 5% on global scale (Sasser & Carter, 1982). In Niger these losses can reach 60% in sweet pepper (Haougui & Kollo, 2006).

Recognizing the importance of RKN in vegetable crops, extensive surveys of nematodes associated with vegetables have been conducted, extensive surveys of vegetable diseases (Sikora, Reckhaus, & Adamou, 1988; Abdou, 2010; Haougui, Domma, Toufique, & Kollo, 2011; Djibey, 2012; Nourh, 2012; Haougui, A., Nouri, Basso, Doumma, & Adam, 2013) and have shown a high infestation level on the fields of farmer. For instance, Sikora et al. (1988) reported that *Meloidogyne incognita* was found on 48% of the vegetable plants examined, with the average level of root galling ranging from 1-10% to 51-100%. An unidentified fungal wilt disease was observed on tomato but the extent of the distribution and importance was not examined in any of the vegetable crops sampled (Sikora, personal communication 2013). Furthermore, Haougui (1999) reported that in vegetable growing sites infested by plant parasitic nematodes, 50% of plants died before fructification and 12.5% wilted.

Recently, in 2011 during routine disease survey on sweet pepper in the growing areas of Diffa and Aguié, severe infestations of RKN were observed with severely galled roots and yellowing of leaves leading to the decline of sweet pepper plants. This disease is becoming more frequent and severe and that could be attributed to the simultaneous combined effect abiotic and biotic stresses. The presence of these pathogens puts sweet pepper production in a significant risk and reducing yield in some cases even results in abandoning fields. The damage inflicted by these pathogens is becoming more frequent, severe and is associated with sudden death syndrome of sweet pepper. It was

suspected that the sudden death of sweet pepper was due to the simultaneous combined effect of abiotic and biotic stresses.

### **Statement of the Problem**

Diseases are responsible for losses of at least 10% of global food production, representing a threat to food security (Strange & Scott, 2005). Climate change including high variance in climate variability may aggravate the situation seriously. In fact it will add uncertainty to the course of the disease evolution and will preempt actual control methods and then rend it difficult to manage the diseases on a sustainable basis (Coakley, Scherm, & Chakraborty, 1999). Climate change could alter the stages and pace of development of pathogens, modify host resistance and result in host-pathogen interactions (Garrett, Dendy, Frank, Rouse, & Travers, 2006). The consequences are likely changes in geographical distribution of host pathogen and a change in the profile of crop losses, caused in part by changes in the effectiveness of control strategies (Coakley et al., 1999).

Intensified research on climate change–related issues could result in improved understanding and management of plant diseases in the face of current and future climate extremes (Coakley et al.). So an effective management of pest and pathogens is a key to effective use of natural resources, and maintaining incomes and benefits for producers by reducing losses and keeping food prices affordable to maintain food security (Garrett et al., 2012). For rural producers to gain these benefits, they require access to productive services (Kongolo, 2012) particularly access to agricultural extension services and climate information in order to use appropriate disease

control strategies. In Niger, sweet pepper decline caused by Nematode, (*Meloidogyne* spp.) and fungus, (*Fusarium* spp.) caused heavy yields losses. In the climate change context, it will expect to have more challenges with crop disease. Changing weather (e.g. temperature, rainfall) can induce severe plant disease epidemics (Coakley et al. 1999). Other limiting factors of low yield are inappropriate agronomic practices and poor farmers' understanding of climate adaptation strategies.

So far, no study has been undertaken to investigate the effect of changes in temperature on the population dynamics of nematodes and the development of the wilt disease (sudden death disease) of sweet pepper plants in Niger. First a farmer-based analysis of climate variability and adaptation options for the management of the disease is needed. Second, understanding the interaction between *Meloidogyne* spp., biotic (*Fusarium* spp.) and abiotic factors (temperature) on the development of the wilt disease in sweet pepper is needed in designing appropriate diseases control and adaptive strategies to climate change.

### **Purpose of the study**

Keeping in view the importance of sweet pepper in Niger and the above mentioned facts, this research is aimed at generating information on, how rural farmers perceive climate change and their access to extension services, the identification, distribution and importance of causal agents of wilt disease on sweet pepper in southern and eastern Niger, and also to find out the possible interrelationship between the biotic and abiotic factors on the development of the sudden death syndrome of sweet pepper and how

increasing temperature affect the pathogen variables. The study therefore seeks to achieve the following specific objectives:

1. To carry out survey to determine the distribution and frequency of occurrence of *Meloidogyne* spp. and *Fusarium* spp. associated with sweet pepper.
2. To identify *Meloidogyne* spp. and *Fusarium* spp. associated with sweet pepper
3. Determine effect of temperature on co-infection of *Meloidogyne* spp. and *Fusarium* spp. on the severity of sudden wilt of sweet pepper.
4. To assess the perception of farmers on climatic variability, agricultural adaptation and access to agricultural extension services

### **Research hypotheses**

**Hypothesis 1:** The “Sudden wilt disease” of sweet pepper is caused by a joint infection of root knot nematodes (*Meloidogyne* spp.) and *Fusarium* spp.

**Hypothesis 2:** The population structure of the pathogens is affected by the local crop practices and climate variability.

**Hypothesis 3:** The interaction between the abiotic factors and the pathogens affects the disease severity.

### **Significance of the study**

Occurrences of wilt diseases in sweet pepper in Diffa and Aguié and prevailing problems associated with changes in weather patterns caused by climate change provide sufficient reason to examine and identify the causal agents of wilt disease and to investigate whether sweet pepper farmers

perceive the variation in weather and what their adaptation strategies are. This study will provide insight into the perceptions and adaptations to climate variabilities in Diffa and Aguié. The findings will generate some policy implications for supporting access to information on appropriate adaptation strategies to climate variability and resistant crop varieties for effective management of the wilt disease in sweet pepper.

The findings obtained after carrying out this study should create the awareness in farmers in Diffa and Aguié on the abundance of *Meloidogyne* spp. and *Fusarium* spp. which will influence their decision on crops to grow and management practices to adopt when growing sweet pepper. Policy makers may use the recommendation from the present study to advise farmers about practices required to reduce disease dissemination. In terms of academic contribution, the study will contribute in the identification of causal agents of wilt disease in sweet. It will have significant implications for epidemiology and management.

### **Limitations of the Study**

Despite the interesting results which this study will present, it is important to recognize the limitations of the method. First, this study relies on farmers' perception of climate and adaptations and access to extensions services. It could have been difficult for most farmers to remember past events due to their limited capacity to record climatic trends over a longer period of time. The perception of farmers may be biased towards a cultural attitude of young towards elders. Young farmers may therefore have felt obliged to give

the same as their elders say all even when in fact they had different (Maddison, 2007).

Another limitation is that the questionnaire used for data collection was prepared in English and most of the farmers were illiterate. Therefore interpreters had to be used to translate the questions into local languages in order for respondents to answer the questions. It is important to be aware of language barriers and semantics, as many differences may be lost in translation (both between sociolects and ethnic languages). For instance, Tschakert (2007) demonstrate that the word climate leads to a plurality of definition in Wolof, Senegal's dominant language. In Niger, there are also many ethnic groups with different languages. Secondly, this used a face to face interview using questionnaire. Non-parametric method like Participatory Rural Appraisal tools (PRA) could be used to examine to capture more the effect of factors having less variation in sampled data.

For the identification of *Meloidogyne* spp., this study used only populations of *Meloidogyne* spp. and *Fusarium* spp. in sweet pepper. Screening a large sample on different host can allow identification of various species present in the study area and determination of genetic diversity among root knot nematodes. The interaction between *Meloidogyne* spp. and *Fusarium* spp. on sweet pepper in this study will use only growth chamber experiment data. It is important to run combining field and controlled experiments jointly that allow better understanding of dynamic processes, in order to identify host parameters more correlated to the physiological changes that occurred on host plant disease.



## **Definitions of Terms**

Here we first provided some definitions.

***Climate change:*** According to IPCC (2007) climate change is defined as a change in the state of the climate that can be identified (e.g. using statistical tests) by changes in the mean and/or the variability of its properties and that persists for an extended period, typically decades or longer. It refers to any change in climate over time, whether due to natural variability or as a result of human activity.

***Adaptations:*** In the specific context of climate change, adaptations are initiatives and measures undertaken to reduce the vulnerability of natural and human systems against actual or expected climate change effects (IPCC, 2007).

***Perception:*** Perception is defined as the process by which we receive information or stimuli from our environment and transform it into psychological awareness. It is interesting to see that people infer about a certain situation or phenomenon differently using the same or different sets of information (Ban & Hawkins, 2000). This varies with the individual's past experiences and present sets or attitudes acting through values, needs, memories, moods, social circumstances, and expectations (Saarinen, 1976).

***The onset of the rainy season:*** The onset was defined as the day after the 1st of May with 20 mm of rain cumulated over 1 or 2 consecutive days without a dry spell of more than 20 days within the subsequent 30 days (Alhassane, Salack, Ly, Lona, Traore, & Sarr, 2013).

*The cessation of the rainy season:* The cessation was defined as the first day after 1st September when the climatic water balance is less or equal to 0.5 mm, such that water consumption of the plant and climatic demand deplete the soil water reserve (Alhassane, Salack, Ly, Lona, Traore, & Sarr, 2013).

*The length of the rainy season:* The length of the rainy season for a particular year is obtained from the difference between cessation and onset of that year (Alhassane, Salack, Ly, Lona, Traore, & Sarr, 2013).

### **Organization of Study**

This thesis is structured into 6 main chapters. Chapter one starts with an introduction (this Chapter) that presents background of the research. The justification, statement of the problem and objectives of the research are presented, and the research hypotheses, significance of the study, limitations of the study, definitions of terms and organization of the study are given. The remaining chapters of the thesis are organized as follows: Chapter two contains a review of the literature, Chapter three describes materials and methods; in Chapter four, the findings of this thesis are presented and the discussions are presented in Chapter five. In Chapter six, the synthesis of findings in terms of conclusions and recommendations are presented.

## CHAPTER TWO

### LITERATURE REVIEW

#### **Sweet pepper cultivation in Niger**

Sweet pepper (*Capsicum annuum* L.) is considered one of the most important vegetables cultivated extensively in Niger especially in the Eastern part. It ranks third after cowpea and onion. In Niger, sweet pepper is grown in open field cultivation for the local market and exportation. The number of farmers involved in producing, transforming or trading sweet pepper has been estimated at 20 000 to 30 000 (Djibey, 2012). It is an important off-season vegetable and offers potential for boosting economy of farmers of Diffa region and Aguié. It is one of the high value crops in Niger having a growing area of approx. 7300 ha with a total mean production of 126,000 t fresh peppers and yield of 17ton/ha yields (Djibey, 2012). The main production area is Diffa Region with more than 85% of the national production. It is grown as a vegetable and consumed both fresh and dehydrated spice and also exported specifically to Nigeria. Its production also improves the livelihood of the smallholder farmers through increased incomes.

The crop therefore constitutes a source of income for a significant number of households in rural, periurban and urban areas, and consequently in their food security, through the use of this income to cover food expenses. To obtain paprika, sweet pepper fruit must be dehydrated by heating and then ground. Traditionally in Niger, paprika is obtained by sun drying which is principally used as an ingredient in a broad variety of dishes.

Sweet pepper fruit is an important source of natural colors and antioxidant compounds (Howard, Talcott, Brenes, & Villalon, 2000; Lee, Howard, & Villalon, 1995) which have high nutritional value for human health. These compounds in food are an important health-protecting factor. They have been recognized as being beneficial for the prevention of widespread human diseases, including cancer and cardiovascular diseases (Sies, 1991).

Despite its importance, sweet pepper growers in Niger face several challenges in open field cultivation, like vagaries of the weather viz. fluctuating temperature and flooding which affect the yield and quality of the produce. The most important factors hindering the production of sweet pepper are pests and pathogens. The major ones causing reduction in yield and quality of sweet pepper include roots knot nematodes (*Meloidogyne* spp.) and *Fusarium* spp. (Haougui, 1999; Haougui & Bizo, 2009).

### **Sweet pepper diseases**

Sweet pepper is liable to be attack by many bacterial, fungal, viral and nematode diseases as well as physiological disorder. However, *Fusarium* wilt is considered the major devastative and destructive disease affecting crop production of pepper (Black et al., 1991; Attia & Abada, 1994).

### **Wilt disease caused by *Meloidogyne* spp.**

The root knot nematode (RKN) *Meloidogyne* spp. are an important plant parasitic nematode that infects different plant species causing significant yield losses either through direct feeding of roots. The infective juveniles (J2)

penetrate into the plant through the roots and migrates into the actively dividing plants cells (Srivastava, 1973).

The development and reproduction of RKN is dependent on whether or not specialized feeding sites within the vascular tissues are induced. If the host is susceptible, the feeding sites are enlarged resulting in giant cells which arise due to repeated cell divisions. Galling occurs due to hypertrophy of cortical cells and within the root galls nematodes continually undergo moulting to the mature female (Jenkins et al., 1995).

The changes that occur in the roots include disruption of the root xylem epidermal and cortical tissues development, which in turn affect water and nutrient uptake resulting in stunted growth (Kirkpatrick et al., 1991). Due to galled roots have only limited ability to absorb and transport water and nutrients to the rest of the plant, severely infected plants may wilt even in the presence of sufficient soil moisture. Plants also may exhibit nutrient deficiency symptoms because of their reduced ability to absorb and transport nutrients from the soil (Mitkowski & Abawi. 2003).

#### **Wilt disease caused by *Fusarium* spp.**

Sweet pepper plants are subject to diseases that cause wilts. The *Fusarium* species attacks the stem of the pepper plant at the soil line and causes a soft decay of the outer tissues. This girdling of the stem causes a wilting and yellowing of the leaves and an eventual drying of the stem and branches (Coons, 1993). The stems of diseased plants become covered with a

white growth of the fungus in which is light-brown bodies (sclerotia) about the size of a mustard seed.

When dead plants are pulled, a clump of fungus-infested soil often stays attached to the roots. The fungus lives for a long time in the soil and requires warm, moist weather for its active development. It seems to be most active in poorly drained, light, sandy soils. The sclerotia can live in the soil for some time and are spread by cultivation or washing rains. Under favourable conditions the bodies can put out fungus threads and infest new areas in the field (Coons, 1993).

#### **Root knot nematodes (*Meloidogyne* spp.)**

Root knot nematodes (*Meloidogyne* spp.) are obligate parasites that infect underground plant organs (Fourie & McDonald, 2000; Anwar, Zia, Hussain, & Kamran, 2007) and are economically important pests of a wide range of vegetables throughout the world (Castagnone-sereno, 2006). They are widely distributed, have a broad-host range in the tropics and cause substantial reduction of crop yield and quality. They attack most of the cultivated plant species and are responsible for crop losses worth billions of dollars annually (Agrios, 2005).

They also attack crops in the greenhouses, when non-sterilized soil is used (Agrios, 2005). More than 80 species have been described (Karssen, 2002); and the vast majority of research has focused on just four species that have commonly been recognized as the major and widely distributed species (Eisenback, Hirschmann, Sasser, & Triantaphyllou, 1981). These occur in the

following order; *M. incognita* (Kofoid & White) chitwood, 47%; *M. javanica* (Treub) Chitwood, 40%; *M. arenaria* (Neal) Chitwood, 7% and *M. hapla* Chitwood, 6% (Sasser, 1980). The reason for the elevated status of these four species is partly due to the fact that they are extremely widespread and infect a wide range of principal crops (Taylor, Sasser, & Nelson, 1982). In that study, the authors reported that *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* made up 99% of all species identified in over 660 isolates from 65 countries.

However, in the recent past, several of studies have clearly demonstrated the enormous impact a number of formerly largely ignored *Meloidogyne* species can have. It seems questionable whether the historic classification into ‘major’ and ‘minor’ root-knot nematode species is still justifiable. More recently, greater attention has focused on *M. enterolobii*, a species originally described from China (Yang & Eisenback, 1983). Various studies (Carneiro et al. 2006; Kiewnick, Karssen, Brito, Oggenfuss, & Frey, 2008; Kiewnick et al. 2009) have reported the presence of this pathogen in vegetable crops considered resistant to *M. incognita*, *M. javanica* and *M. arenaria* (Neal, 1889; Chitwood, 1949). Due to its aggressiveness (Hussey & Janssen 2002), this pathogen has the ability to overcome the resistance of tomato and pepper genotypes.

#### **Life and disease cycle of *Meloidogyne* spp.**

The adult females deposit eggs into an egg sac, which is made up of a gelatinous matrix produced by its rectal glands (Bird & Rogers, 1965). Under favourable conditions mobile second-stage juveniles (J2) emerge from the

eggs locate host plants and attack nearby roots (Figure 1). The J2s feed on protoxylem and protophloem inside the root and inject secretions into the root cells; incite the production of multinucleate giant cells, which provide a continuous sole source of nutrition for the rest of its life cycle. At the same time, galling of the roots occurs. After the feeding site has been established, J2 increase in size and undergo subsequent moults into third- and fourth-stage juveniles (J3 and J4), and after a fourth and final moult into adult females or males. The J2 (the infective stage) and males are the stages of *Meloidogyne* spp. that can be found freely in soil.

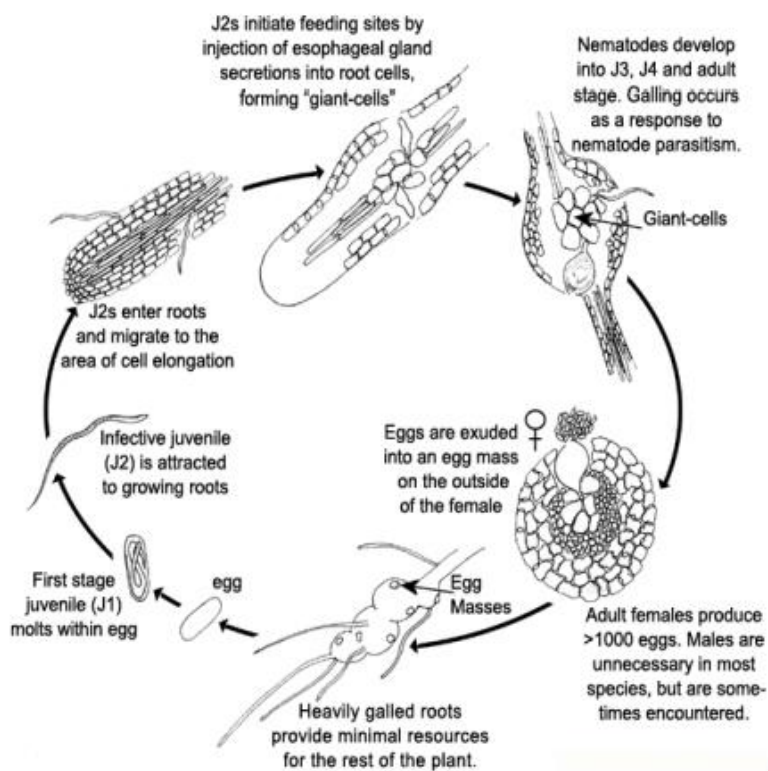


Figure 1: Life and disease cycle of root-knot nematodes (*Meloidogyne* spp.).

Source of the figure: The American Pathological Society (2003)



### **Root knot nematode disease symptoms**

The presence of galls on the root system is the most evident diagnostic symptom caused by root-knot nematodes. Symptoms of the disease which appear on the underground parts of infected hosts include abnormal swellings (knot or root-galls), lesions, and rotting of roots (Agris, 2005; Pajovic, Sirca, Geric, Stare, & Urek, 2007). Furthermore, deformation and blockage of vascular tissues at the feeding sites limit translocation of water and nutrients, which further suppress plant growth and crop yield (Anwar & Van Gundy, 1993; Hussy & Williamson, 1997). Consequently, plants with galled roots are less able to accumulate water and nutrients efficiently, thus are more susceptible to drought, heat stress and nutrient deficiencies (Taylor, 2003). Other symptoms of the disease include, yellowing of leaves, stunting growth, wilting and patches in the field and decline in fruit yield, quality and quantity (Sikora & Fernandez, 2005).

### **Root Knot Nematodes (RKN) associated with sweet pepper in Niger**

Root-knot disease caused by *Meloidogyne* spp. is among the most widespread crops disease in the World. In Niger, information on RKNs associated with sweet pepper is limited. The first works published on RKNs associated with sweet pepper were from a general nematode survey carried out by Sikora et al. (1988). The authors reported that *M. incognita* was found in 86% of the vegetable areas sampled and sweet pepper was one of the most severely galled. Haougui (1999) and Djibey (2012) investigated the presence of RKNs and other plant parasitic nematodes on vegetables crops and reported

respectively the presence of 13 and 12 genera of nematodes and were associated with sweet pepper. Among these plant parasitic nematodes, the root knot nematodes were predominant in Diffa District and *M. javanica* was the most frequently occurring species at all surveyed sites. In other similar studies, Haougui et al. (2013) underlined the occurrence of RKNs in pepper crops with population density up to 4500 J2s per gram of roots in Aguié district.

Around the world, several studies have been carried on nematodes associated with sweet pepper. For instance Khan (2008) reported that RKNs, *M. incognita* and *M. javanica* were the most damaging and widespread of all the nematode pests of chili and sweet pepper in the savanna zone of northern Nigeria. Dalhatu, Khan and Umar (2014) and Bachie (2015) found a high population of *M. incognita* infesting bell pepper (*Capsicum annuum*) in southern California. Kiewnick, Karssen, Brito, Oggenfuss and Frey (2008) reported that *M. enterolobii* was reproduced on pepper cultivar carrying the N resistance gene to RKNs.

#### **General morphology of *Meloidogyne* spp.**

The general morphological of the genus *Meloidogyne* has been based primarily on light microscope observations of morphological characters and morphometrics of females, males and second-stages juveniles (Figure 2) (Eisenback & Hunt, 2009).

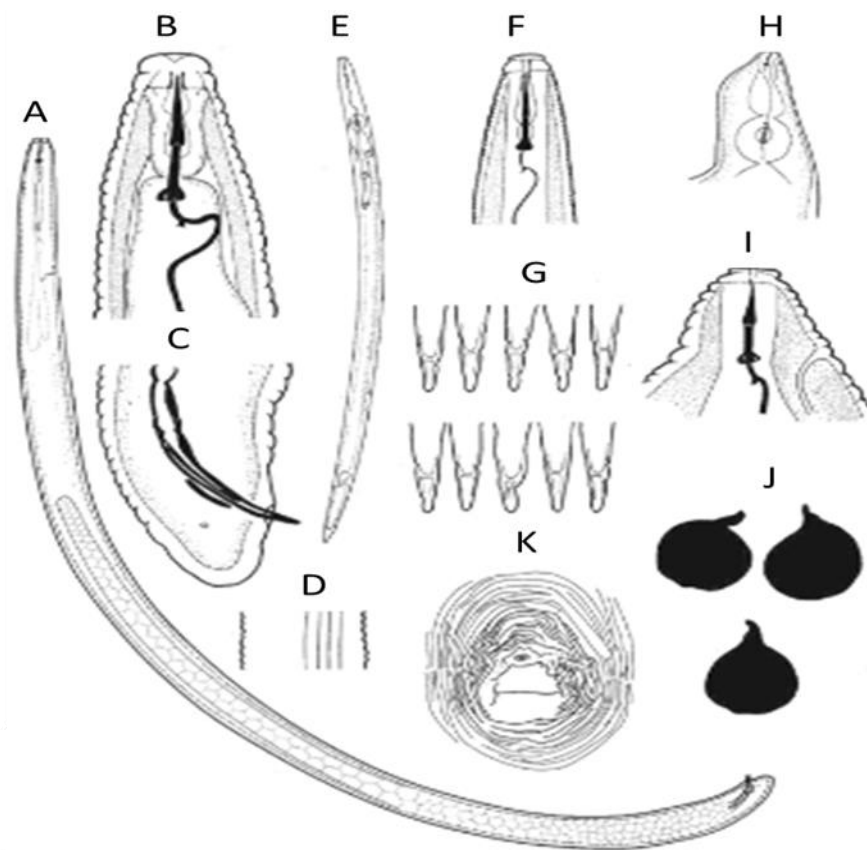


Figure 2: Example of line drawings of (*Meloidogyne chitwood*) appropriate to illustrate the description of a new species. A: entire male; B: male anterior region; C: male tail region; D: male lateral field, E: entire J2; F: J2 anterior region; G: J2 tail region; H- I: female anterior region, lateral view; J: entire female; K: perineal pattern (Jepson, 1985).

The females are endoparasitic and annulated, pearly white and globular to pear shaped, 400–1300  $\mu\text{m}$  long, 300–700  $\mu\text{m}$  wide and have lateral fields each with 4 incisures. The stylet is dorsally curved, 10–25  $\mu\text{m}$  long, with rounded to ovoid stylet knobs, set off to sloping posteriorly. The basic feature of adult females is the perineal pattern, which is located in the posterior body region. The males are vermiform, annulated, migratory, slightly tapering anteriorly, bluntly rounded posteriorly, 700–2000  $\mu\text{m}$  long and 25–45  $\mu\text{m}$  wide.

The stylet is 13–30  $\mu\text{m}$  long, with stylet knobs, variable in shape. The second-stage juveniles are vermiform, annulated, infective and migratory, tapering at ends, 250–700  $\mu\text{m}$  long, 12–18  $\mu\text{m}$  wide, tail length 15–100  $\mu\text{m}$  and hyaline tail part 5–30  $\mu\text{m}$  in length (MacLeo & Baker, 2003).

The morphological and morphometrical characters most commonly used to separate *Meloidogyne* species are presented in Table 1 (Hunt & Handoo, 2009).

Table 1- *Morphological characters used for Meloidogyne species identification*

<i>Meloidogyne</i> spp.	Morphological characters
Females	shape of body stylet length shape of stylet cone, shaft and basal knobs nature of perineal pattern, form of dorsal arch
Males	size, height and shape of labial cap stylet length shape of stylet cone, shaft and basal knobs distance of the dorsal gland orifice from the stylet spicule length and shape
Second-stage juveniles	body and stylet length shape of stylet basal knobs distance of dorsal gland orifice from the stylet base length of the tail and hyaline terminus

### **Intraspecific variation in *Meloidogyne* species**

Even though overall morphological enable the identification of *Meloidogyne* species, intraspecific variability is an important aspect of nematode biology that needs to be taken into account to avoid misidentifications and to optimize management strategies. For *Meloidogyne*, host races are considered intraspecific variants that can be separated by their ability to reproduce on a set of host plants from different genera.

To describe intraspecific variation in pathogenicity in *Meloidogyne* spp., two concepts are used. The best known is the host race concept, which is based on host plant differentials (Hartman and Sasser, 1985; Sasser and Triantaphyllou, 1977). Differential host tests are used to detect mixed nematode populations consisting of more than one species and to distinguish host races (Hartman and Sasser, 1985). The test differentiated among *M. incognita* (Kofoid & White) Chitwood host races 1, 2, 3, and 4, *M. javanica* (Treub) Chitwood (without host race specification), *M. hapla* Chitwood (without host race specification), and *M. arenaria* (Neal) Chitwood host races 1 and 2, according to their reproduction on specific cultivars of cotton, tobacco, pepper, watermelon, peanut, and tomato. The second way of classifying intraspecific variation, by biotypes, is based on responses of populations of a *Meloidogyne* species to more than one genotype of one host plant species.

Roberts (1995) applied and elaborated this concept to the *M. incognita*-tomato relationship.

## **Identification of Root Knot Nematodes (RKNs)**

### **Morphological identification of Root Knot Nematodes**

Accurate identification of plant parasitic nematodes is of significant importance for developing an effective control measures. Generally RKNs have been identified based on morphological characters (Eisenback & Triantaphyllou, 1991). Chitwood (1949) was the first to identify and describe *M. hapla* and *M. incognita* var. *acrita* on the basis of morphological characters. Chitwood (1949) also redescribed *M. arenaria*, *M. exigua*, *M. incognita*, and *M. javanica* mentioned that genus *Meloidogyne* was extremely adaptable and their morphological characters showed considerable variations.

Furthermore, based on morphological characters, 23 species have been described by Whitehead (1968) and 51 by Jepson (1987). Since then, several methods have been developed to identify RKNs including isozyme analyses (Esbenshade & Triantaphyllou, 1990) and host-plant response (Taylor & Sasser, 1978; Hartman & Sasser, 1985). Identification of *Meloidogyne* spp. based only on these classical approaches use of morphology and morphometrics, isozyme profiles and differential host test, is to some extent inaccurate, unreliable and laborious (Onkendi & Moleleki, 2013).

### **Molecular identification of Root Knot Nematodes**

#### ***Polymerase chain reaction (PCR) based techniques***

To overcome the challenges in morphological identification, DNA-based diagnostic has been used (Powers & Harris, 1993; Powers et al., 1997) and gives accurate identification of the genus *Meloidogyne*. PCR based techniques are a strong diagnostic tool for accurate detection of pathogens and

have also been widely used for the identification of nematodes (Zijlstra, Lever, Uenk, & van Silfhout, 1995; Zijlstra, Donkers-Venne, & Fargette, 2000; Adam, Phillips, & Blok, 2007). Combining both the morphological and molecular approaches gives high accuracy in nematode identification (Tigano, Carneiro, Jeyaprakash, Dickson, & Adams, 2005). For instance, some authors (Brito, Powers, Mullin, & Dickson, 2004; Iwahori, Truc, Ban, & Ichinose, 2009; Zhuo, Hu, Liao, & Rui, 2010) successfully identified *M. enterolobii* based on PCR amplification and obtained approximately 705~bp band using C2F3/1108 primers.

#### ***PCR-RFLP based diagnosis***

Restriction Fragment Length Polymorphism (RFLP) is a fast technique which can be used to distinguish species of nematodes, either the differentiation of species or populations within species (Dalmaso, 1993; Fargette, Phillips, Blok, Waugh, & Trudgill, 1996). The PCR products obtained from different species can be digested by restriction enzyme and the products of digestion are separated by electrophoresis followed by visualization of banding pattern. Restriction of mtDNA with *Hinf I* enzyme enabled the identification of important *Meloidogyne* spp., isolates and host races (Harris, Sandall, & Powers, 1990) suggesting that mtDNA has considerable potential for species identification (Zijlstra et al., 1995; Schmitz et al., 1998).

Powers and Harris (1993) classified five RKNs (*M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla* and *M. chitwoodi*) based on PCR-RFLP method. For instance in Pakistan, PCR-RFLP provided discriminatory profiles

useful for two RKNs spp. *M. incognita* and *M. javanica* (Mohamad, 2014). The two species were easily differentiated by the size of the PCR products (1.7 kb fragment in PCR amplification). On the other hand, Powers et al. (2005) reported a PCR product of the two species of 1.5 kb length and *Hinf I* digestion of the 1.5kb generated two fragments for *M. incognita* but none for *M. javanica*.

### **Effect of temperature on population dynamics of *Meloidogyne* spp.**

Changing of climatic factors (temperature and rainfall) are likely the main factors that determine the distribution and population dynamics of pathogens in a natural ecosystem. Temperature influences all aspects of the life cycles and behaviour of nematodes, including hatching, mortality, invasion and development (Evans & Perry, 2009).

*Meloidogyne* species vary in the temperature range over which hatching occurs; the optimal temperature for hatching is generally indicative of the geographic region or seasonal preference of their plant hosts (Lee & Atkinson, 1976).

Ploeg and Maris (1999) studied the effect of temperature on the duration of the life cycle of a *Meloidogyne incognita* population. They revealed that they completed their life cycle on tomato at average soil temperature between 16.2 and 30°C. Khan and Ashraf (2005) studied life cycle *Meloidogyne incognita* and found that it required 32 days to complete their life cycle on lettuce at a temperature ranging between 18- 25°C.



The effect of temperature on hatch and activity of second-stage juveniles of the root-knot nematode was carried out by Morris, Horgan, Downes, Christine and Griffin (2011). They found that when egg masses were incubated at constant temperatures, J2 hatched between 15 and 25°C, with limited hatch (< 1%) at 10 and 30°C. Tsai (2008) studied the effect of temperature on the survival of *Meloidogyne incognita*. He pointed out that at 35°C, all the nematodes were killed after 60 days, only few nematodes survived at 30°C after 25 days.

### ***Fusarium* spp.**

*Fusarium* species are among the most ubiquitous fungi in terrestrial ecosystems (Ploetz, 2006). Since its identification by Link (1809), the genus *Fusarium* has been one of genera containing many recalcitrant plant-pathogenic species that are not easy to differentiate and control. Its members are distributed across the globe where they are responsible for huge economic losses due to reductions in harvest yields and/or the quality of staple foods (Leslie & Summerell, 2006).

Most of members of this genus are significant plant pathogens, based on the diversity of hosts they attack, the number of pathogenic taxa and the range of habitats in which they cause disease in several agriculturally important crops. More than 80% of all cultivated plants are associated with at least one disease caused by *Fusarium* species (Leslie & Summerell, 2006).

The most important plant pathogens in *Fusarium* genus are of four major groups, generally (Aoki, O'Donnell, & Geiser, 2014). These are the *F. fujikuroi* species complex, *F. graminearum* species complex, *F. Oxysporum*

species complex and *Fusarium solani* species complex. Several *Fusarium* species cause vascular wilts or root rots in many important vegetable (Kraft, Burke, & Haglund, 1981; Nelson, Horst, & Woltz, 1981). Some cause destructive disease on cereal grains (Parry et al., 1995; Goswami & Kistler, 2004), while in the recent past, reports indicate that *Fusarium* is a significantly emerging human pathogen in immunocompromised patients (Walsh & Groll, 1999; Roilides, Dotis, & Katragkou., 2007).

In Solanaceae, *Capsicum* L., wilt is caused by *F.annuum* (Leyendecker & Nakayama, 1956), fruit and stems rot and wilt by *F. oxysporum* (Alfieri, Langdon, Wehlburg, & Kimbrough, 1984), and damping-off by *Fusarium* sp. (Raabe, Connors, Martinez, 1981). More than 80 known formae special that show specific pathogenicity to particular crops, cause vascular wilt, and root and crown rot. On the other hand, non-pathogenic forms of *F. oxysporum* are known to reduce the impact of vascular wilt pathogens. For instance, there are soils particularly suppressive to *Fusarium* wilt diseases in which the non-pathogenic *F. Oxysporum* plays an important role (Alabouvette & Couteaudier, 1992; Tamietti, Ferraris, Matta, & Abbattista- Gentile., 1993).

### ***Fusarium* spp. associated with sweet pepper**

In Niger, little is known about fungi associated with sweet pepper. Haougui et al. (2013) examined the presence of fungi on sweet pepper plants with brown discoloration on roots and the stem and severe wilting symptoms. In their work, they identified *F. oxysporum* as associated with this symptom in sweet pepper. Survey on the investigation of fungi has also been studied in

other countries. Lamb, Roskopf and Sonoda (2001) have reported that *F. solani* was the causal agent of stem and fruit rot in Greenhouse peppers. *F. solani* caused stem and fruit rot as well as wilting and death of the upper portions of the plant that resulted in severe yield losses. Niger, wilting of vegetable crops including sweet pepper caused by *Fusarium* was also reported by Haougui et al. (2013). The authors found *F. oxysporum* was identified as the causal agent of the wilting disease.

The disease also was subsequently found in Ontario (Canada) in the greenhouse sweet pepper where losses reached up 50% (Jarvis, 1994). In New Zealand the same disease on sweet pepper was also observed (Tyson, 2001) and in Korea (Jee et al., 2005). A similar disease was also recently reported in greenhouses in Trinidad where the disease incidence was estimated to be 80% with a yield loss of 40 to 60% (Ramdial & Rampersand, 2010). In Almeria, Spain, were observed wilting symptoms without yellowing of leaves and stunting of sweet pepper plants grown in nursery with *F. oxysporum* f. sp. *capsici* identified as the causal agent (Lomas-Cano ., 2014).

### **Macroscopic and microscopic features of *Fusarium* species**

Morphological characters are by far and away the most commonly used criteria for identifying *Fusarium* species. Most *Fusarium* species grow rapidly on Sabouraud dextrose agar at 25°C and produce woolly to cottony, flat, spreading colonies. The colour of the colony may be white, cream, tan, salmon, cinnamon, yellow, red, violet, pink or purple (Figure 2); and on the reverse, it may be colourless, red, dark purple, or brown (Kontoyiannis et al., 2000).

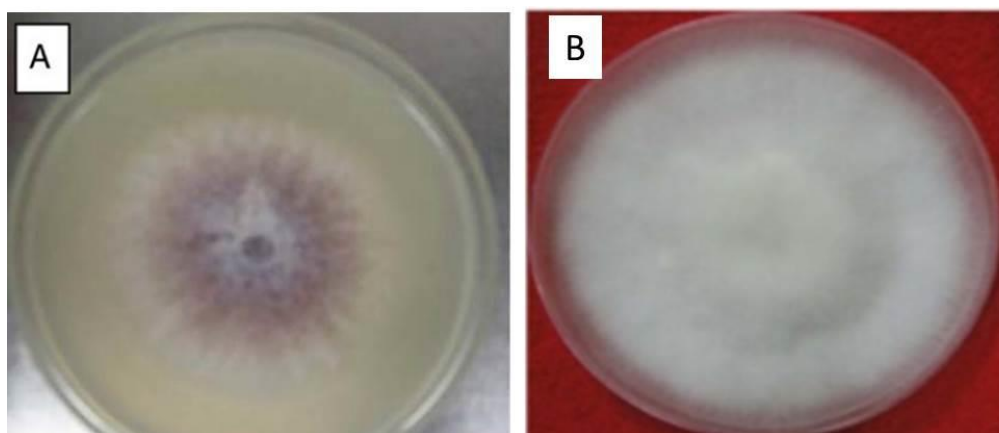


Figure 3: Type of colonies produced by *Fusarium* species: A -*Fusarium oxysporum* (Bayona et al., 2011), B-*Fusarium solani* (Jeon et al., 2013)

*Fusarium* is characterized by the production of slimy, hyaline, septate, macroconidia that in most species are produced in fruiting-structures called sporodochia. Some species also produced distinctly conidia in the areal mycelium often referred as microconidia. According to the species and/or the ecological situation, macroconidia or microconidia dominate on the natural substrate (Seifert, 1996).

The shape of the macroconidia (Figure 4) often is given the greatest weighting when defining species, but differences in macroconidial shape and size can be confusing, subjective, and dependent upon the environment in which they are produced. Other spores, e.g., microconidia and chlamydospores (Figure 5), also are important in morphological species definitions (Leslie & Summerell, 2000).

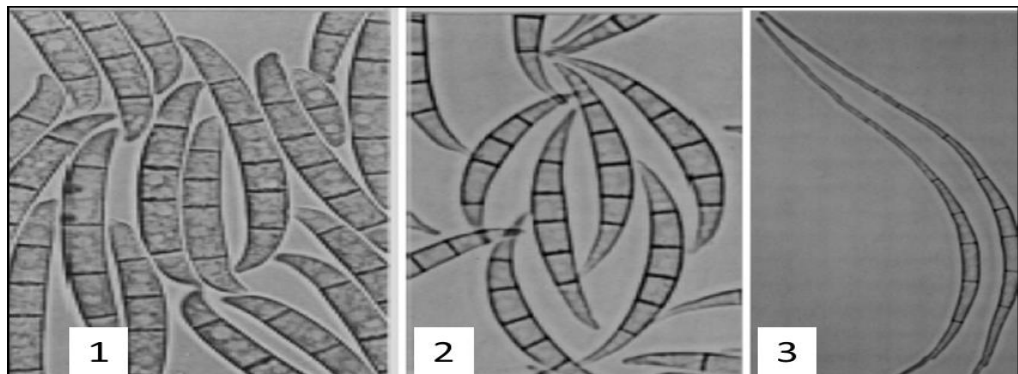


Figure 4: Hyaline septate macroconidia of *Fusarium solani* (1), *Fusarium equiseti* (2), *Fusarium longipeps* (3) (Nelson et al., 1994).

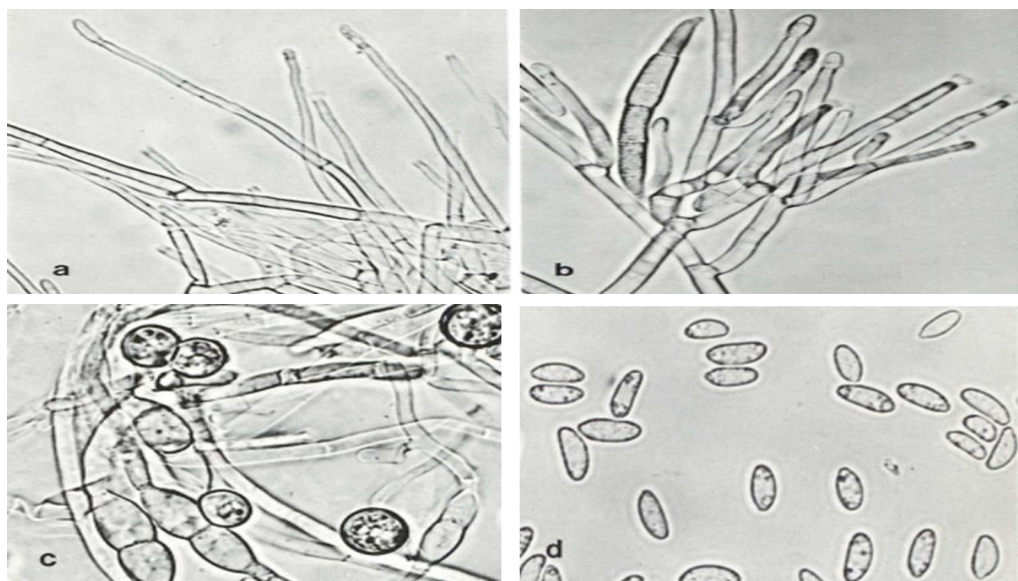


Figure 5: *Fusarium solani*: a-b conidiospores; c chlamydospores, d microconidia (x1000) (Nelson et al., 1994).

Macroscopic and microscopic features such as length and shape of the macroconidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are key features for the differentiation of *Fusarium* species.

### **Variability in *Fusarium* species**

The genus *Fusarium* consists of populations that are quite variable. For this reason, identification of its different species requires special culture media and methods, as well as standard incubation conditions. High variability in species, especially under different environmental conditions, has caused taxonomists to consider some special criteria to be important in the classification of species. For this reason, different methods and keys have been presented for the identification of the species (Booth, 1975). The large variation in some of the characteristics of *Fusarium* isolates such as pathogenicity, colony morphology of the colony, e.g. form and colour, mode of development of the chlamydospores, existence or lack of sporodochia and even the type of microconidia, have resulted in different emphasis on character and different classification of species into intra-specific groups.

The use of sub-species in the classification of *Fusarium* makes it possible to distinguish between the populations with major ecological and physiological differences, but with no major morphological differences (Sangalang, Summerell, Burgess, & Backhouse, 1995)

Most of the intra specific classifications are based on pathogenic behaviour and vegetative compatibility groups (VCG). Some researchers have placed some isolates of VCG into the same race using the pectic zymogram technique (Elias, & Schneider, 1991). Others have classified different races according to special geographic region and many have used different molecular markers for distinction among species (Pegg, Shivas, Moore, & Bentley, 1995). Many fungi, including the *Fusarium* species, release extra-

cellular enzymes which break down the pectin of the cell walls of many plants. Pectic enzymes of pathogens cause root rotting and wilting of plants. Different patterns resulting from pectic enzymes have been studied in different *Fusarium* isolates (Szeczi, 1990a; Szeczi, 1990b).

### **Identification of *Fusarium* spp.**

#### **Morphological identification of *Fusarium* spp.**

*Fusarium* is taxonomically complex genus. The characterization based on morphological features of *Fusarium* species is often difficult due to the variability between isolates. Some diagnostics can be performed quickly when the disease is recurrent or symptoms are well known (Jarvis, 1977). Traditionally the identification of *Fusarium* species is mainly based on colony morphology, the characterization of spores, the presence or absence of the teleomorph and other cultural characteristics (Leslie & Summerell, 2006). This diagnosis only based on these criteria is a long process (Geiser et al., 2004; Nirenberg & O'Donnell, 1998), which is mostly inaccurate and unreliable. For example, morphologically, *F. oxysporum* can be confused with *F. solani* (Booth, 1971; Nelson et al., 1981).

#### **Molecular identification of *Fusarium* spp.**

To overcome the challenges in morphological characterization, currently, the conventional PCR techniques which can make use of the internally transcribed spacer region (ITS) of the ribosomal DNA (rDNA) is used for limited application (Atallah & Stevenson, 2006). Zhang et al. (2006) and O'Donnell et al. (2008) have studied phylogeny of *F. solani* species

Complex (FSSC) that cause infection in both humans and plants based on three genes of the ribosomal DNA.

Another tool for reliable identification based on sequence information of the translation elongation factor 1 $\alpha$  (TEF-1 $\alpha$ ) gene was developed for the numerous *Fusarium* spp. The translation elongation factor 1- $\alpha$  (TEF) gene, has the highest phylogenetic utility for the identification of *Fusarium* spp. (Geiser et al., 2004). In addition to symptom analysis, and morphological features, molecular-based techniques play a major role in quick, reliable and accurate identification of fungal pathogens (O'Donnell, Kistler, Cigelnik, & Ploetz, 1998). *F. solani* was successfully identified using ITS region-based (Zarrin, Ganj, & Faramarzi, 2016) and based TEF- $\alpha$  region (Nitschke, Nihlgard, & Varrelmann, 2009).

### **Effect of temperature on population dynamics of *Fusarium* species**

There is increasing evidence that overall activities of soilborne fungi are mainly controlled by temperature, water potential and their interactions in soil (Cooke & Whipps, 1993, Killham, 1994).

A study conducted on population dynamics of five *Fusarium* species at three levels of temperatures (13-18/19-24/25-30°C) by Saremi and Burgess (2000) found that temperature had a significant influence on the population level of all test *Fusarium* species. They noted that the population of *Fusarium solani* and *Fusarium compaction* was higher at high temperatures.



### ***Meloidogyne-Fusarium* disease complex**

In nature, plants are simultaneously exposed to a combination of biotic and abiotic stresses that limit the yields of crops (Ramegowda & Senthil-Kumar, 2015). Atkinson (1892) reported for the first time the interaction of root-knot nematode, *Meloidogyne* spp. with *Fusarium* wilt of cotton. He pointed out that in the presence of root knot nematodes and *Fusarium* spp. wilt resistant cotton varieties became more susceptible. Other studies involving fungi and nematodes have been carried out. Guava decline has been associated with co-infection of *M. enterolobii* and *F. solani* that caused major direct losses in Brazil (Gomez, Souza, Mussi-Dias, Silveira, Dolinski, 2011).

Many studies have shown that the disease severity is more intense when two or three pathogens affect the plant at the same time. The establishment of *Fusarium* spp., *Rhizoctonia* spp. and *Pythium* sp. through wounds caused by nematodes, accelerates the degradation of plant phloem and thus form diseases complex (Messiaen, Blancard, Rouxel, Lafon, 1991; Whitehead, 1998). Other researchers have indicated that infection of *Meloidogyne* spp. in the presence of some telluric pathogens resulted in considerable damage on the host plant than either pathogen acting alone (France & Abawi, 1996, Mohamed, Shohla, EL-Eraki, & EL-Gindi, 1990).

Commonly, *M. incognita* reacts with *Fusarium* spp. and creates a complex parasitism which causes more severe symptoms in plants than the action of both pathogens separately (Jeefers & Roberts, 2003). Starr, Jeger, Martyn and Schilling (1989) had earlier studied the interaction of *M. incognita* and *F. Oxysporum f. sp. vasinfectum* on cotton. They found that both

pathogens had effect on cotton mortality, but no interaction was observed at lower nematode populations or at higher populations of *Fusarium*. It is evidently clear from other studies involving disease complexes that there is synergistic interaction between the pathogens involved (Griffin & Thyr, 1988; Siddiqui & Husain, 1992; Siddiqui & Mahmood, 1997; Abdel-Momen & James, 1998; Ganaie & Khan, 2011; Dania, Sheila, & Safiuddin, 2014).

### **Perception of farmers of climate change and agricultural adaptation strategies**

Climate is the main driver of agricultural production. Agriculture is the main source of income for the majority of the developing world, employing about 60% of the workforce and contributing an average of 30% of gross domestic product (GDP) in Sub-Saharan Africa (World Bank, 2013). Climate is the main driver of the agriculture sector and it is likely to affect agricultural production, as agriculture sector is inherently sensitive to climate conditions and is one of the most vulnerable sectors to the risks and impact of global climate change (Parry, Rosenzweig, & Livermore, 2005).

Agriculture is key asset for poor people, providing multiple economic, social, and risk management functions. For example, agriculture contributed to an average of 30% of gross domestic product (GDP) in Sub-Saharan Africa (World Bank, 2013). However, most climate models used to predict climate change scenarios indicated an increase in temperatures for most of African countries (Tadross, Hewiston, & Usman, 2005). These changes of weather are exacerbating existing vulnerabilities of the poorest people who depend on semi-subsistence agriculture for their survival (Slingo, Challinor, Hoskins, &

Wheeler 2005; Morton, 2007). Key variable is awareness about climate change and adaptation methods that is, whether farmers are aware about climate change and various adaptation techniques that they can take to cope with. This type of evidence has led to interest in how farming systems cope and adapt to climate variability (Adger, Arnell, & Tompkins, 2005; Ziervogel, Bharwani, & Downing, 2006).

Therefore farmers need to recognize the climatic changes already taking place in their areas and undertake appropriate investments towards adaptation (Komba & Muchapondwa, 2012). Thus, there is a growing demand from policy makers for a better understanding of the most vulnerable livelihoods (Tschakert, 2007) in order to design appropriate adaptation tools.

The perceptions of the indigenous people about climate change and their responses to climate change have significant roles to play in addressing the negative effects climate change (Obayelu, Adepaju, & Idowu, 2013). In this respect, several studies (Maddison, 2007; Gbetibouo, 2009; Moyo et al., 2012; Okonya, Syndikus, & Kroschel, 2013; Simelton et al., 2013) noted that the success of any adaptation strategies would depend on a better understanding of farmers' perception about climate change and variability. Such information on local farmers' perception cannot be estimated by models (Okonya et al., 2013).

Thus, a number of studies have been done in recent years to understand farmers' perceptions on climate change and climate variability. For instance a study conducted by Sanfo, Lamers, Mueller and Fonta (2012) in Burkina Faso revealed that farmers understand climate change and climate variability

primarily based on weather-crop interactions and on events that are associated with climatic fluctuations. Kemausuor, Dwamena, Bart-Plange and Kyei-Baffour (2011) carried out a study on farmers perceptions on temperature and rainfall amount changes and reported that more than 80% of farmers believe that temperature in Ejura-Sekyedumase district had become warmer and over 90% were of the opinion that rainfall timing had changed, resulting in increased frequency of drought. In fact Gbetibouo (2009) reported that more than 70% of farmers perceived that rainfall had decreased.

Moreover various studies have shown the awareness of farmers on climate change (Maddison, 2007; Fisher, Chaudhury, & McCusker, 2010; Ndambiri et al., 2012; Shashidhra & Reddy, 2012). However, those studies on perceptions of climate dealt with temperature and rainfall patterns (Deressa, Hassan, Alemu, Yesuf, & Ringler, 2009; Fisher et al., 2010). Historical climatic data are often used to confirm the perception of farmers (Orlove, Chiang, & Cane, 2000; Vedwan & Rhoades, 2001; Deressa et al., 2009; Fisher et al., 2010) or to reject them (Maddison, 2007).

Furthermore those studies provided bulk information at country or regional level. So far, not much is known about how local farmers perceive climate change within the same country and what the disparities of skills among farmers are, and assessments whether these perceptions are in line with meteorological observations measurements. Adaptation is place-based and needs the use of place specific strategies (Lobell et al., 2008; Deressa et al., 2011). A comparative approach to understand their perception at local level is useful in revealing the differential effects of climate. It helps also in

underscoring the gaps of adaptive strategies and skills among them and their needs and barriers that prohibit from effectively adapting.

### **Access of small scale farmers to agricultural extension services**

Many farmers in rural areas do not have access to information on how to grow crops or pest and disease management. Empowering farmers with skills and knowledge is a catalyst for them to respond to the effects of climate change, increase crop production damage by pest and disease, and is also a key to sustainable crop production. Agricultural extension is a relevant aspect of agricultural education mostly known in improving their knowledge of new techniques. Hedjazi, Rezaee and Zamani-Miandashti (2006) reported that agricultural extension service is mainly responsible for creating awareness among farmers and has a strong reliance on exchange of information among farmers. According to Idachaba (1987), among other factors that impede the agricultural sector in many developing countries is the lack of feedback from farmers to ensure relevance of the research results presented.

This could be overcome by assessing how best farmers have access to relevant information from extensions agents. Hence, some studies (Mmbengwa, 2009; Anaeto et al., 2012; Mmbengwa, Groenewald, van.Schalkwyk, & Sebopetsa, 2012; Ayanwuyi, 2013) have underlined further the important role of extension services in supporting farmers to adapt to climate change.

## CHAPTER THREE

### METHODOLOGY

#### Description of study sites

The study was carried out in two districts of Niger Republic, Diffa and Aguié, because they are the major pepper producing areas and are in different agro ecological zones (Figure 6).

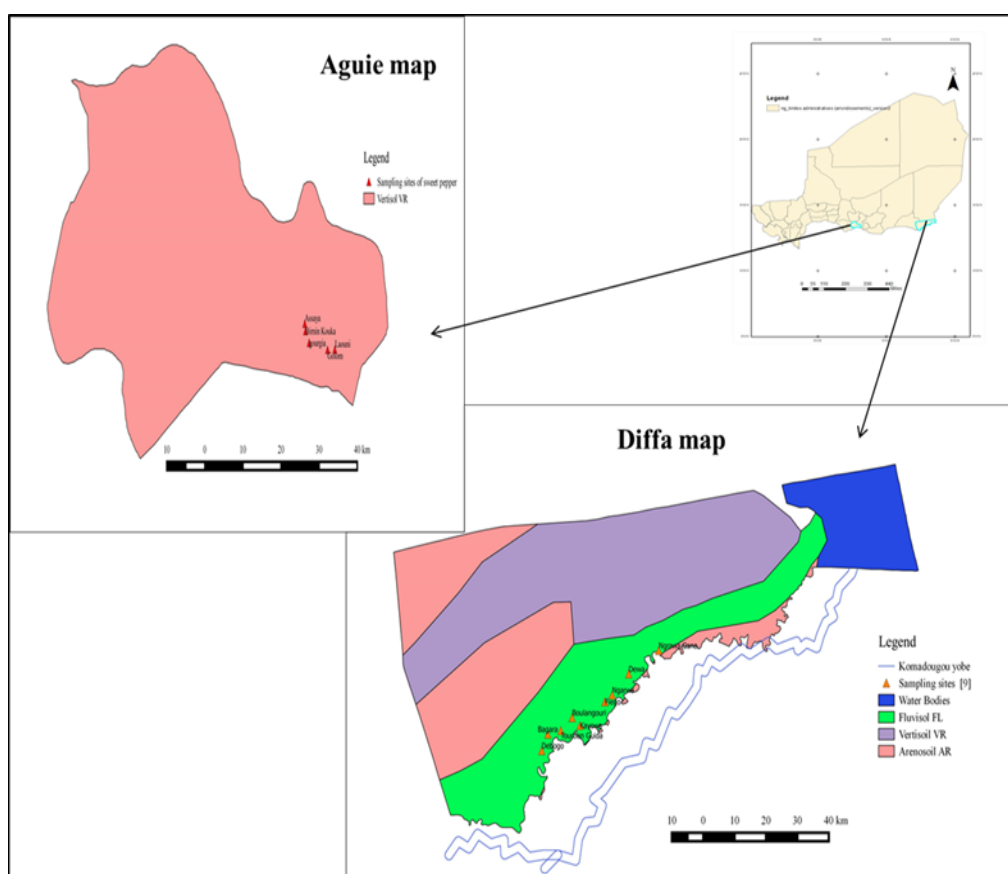


Figure 6: Map showing the two sampling Districts

The mean annual rainfall in Diffa ranges from 200 to 400 mm. The dry season is characterized by the lowest temperatures (average 21°C) between November and February and highest between March and June. The maximum

temperature ranges between 33°C during the rainy season and 46°C during the dry season with a short rainy season mostly less than three months. Aguié district lies between 400 and 600 mm isohyets with the rainy season lasting 3 to 4 months (Djariri, 2009). Temperatures can reach 45°C in the warm dry season and drop to 10°C in the cold dry season. Average temperatures are between 27 and 29°C. Rainfed farming is the main form of crop production in both districts. However it is practiced at a risk due to the large interannual rainfall variability. This activity is a subsistently based on cereal growing (millet, sorghum and maize) and some drought resistant pulse crops (cowpea, groundnut). Despite the fact that local early varieties of pearl millet (*Pennisetum glaucum*) (80-90 days cycle) or (60 days cycle) are cultivated, pearl millet farming has progressively receded, while irrigated crops persist surrounding the Lake Chad and in the Komadougou Yobe valley (Diffa) and Goulbi valley (Aguié).

Given the scarcity of rainfall recently, now the irrigated farming system has always been of crucial importance in generating much of the income in both Diffa and Aguié Districts. Generally, irrigation systems are recession flood systems where the pumps are placed on the border of the river and water is distributed to the different plots by a system of narrow earthen canals. The main irrigated crops are onions, local eggplants, barley, tomato, chili pepper and sweet pepper. Of these crops, irrigated sweet pepper has become the high value crop in the region, ensuring self-sufficiency and generation of income in these areas.

## **Survey for the determination of the distribution and occurrence of *Meloidogyne* spp. and *Fusarium* spp. in sweet pepper in Diffa and Aguié**

### **Sampling and extraction of nematodes**

The sampling was carried out during 2013-2014. Samples were collected in the two agro-ecological zones: eastern Niger (Diffa) and central southern Niger (Aguié). A total of 199 samples of soil and roots were collected randomly from sweet pepper fields in both locations (122 samples in Diffa and 77 samples in Aguié). Sampling was done on both apparently healthy and diseased plants and was collected following the zigzag method (Barker, 1985). Each sample was composed of five sub-samples taken in the rhizosphere of plants at depth of 15-20 cm with a garden trowel. Sampling sites and number of samples for each site are presented in Table 2.

Diagnosis, incidence and severity of the nematode infection were carried out in the phytopathology laboratory at Regional Centre AGRHYMET Niamey, Niger.

### **Extraction of nematodes from soil and roots**

Nematodes were extracted from soil and roots by the Seinhorst method (Seinhorst, 1962a).

For soil nematodes, 500g of soil from each sample were thoroughly mixed in an aliquot of 2 litres of water then macerated carefully for 1 min. After decantation, the nematode suspensions were then passed through a 45µm mesh sieve. The content in the sieve was subjected to active passage through Kleenex paper at room temperature for 48 hours.



Table 2-Sampling sites, number of samples and agroecological zones

Districts	Village	Number of samples	agroecological zones
Diffa	Dewa	10	Eastern Niger
Diffa	Ngarwa	15	Eastern Niger
Diffa	Ngarwa Gana	16	Eastern Niger
Diffa	Fiego	15	Eastern Niger
Diffa	Boulangouri	10	Eastern Niger
Diffa	Kawaya	16	Eastern Niger
Diffa	Dabago	15	Eastern Niger
Diffa	Tourban Guida	15	Eastern Niger
Diffa	Bagara	10	Eastern Niger
Aguie	Gourgia	18	Southern Niger
Aguie	Assaya	16	Southern Niger
Aguie	Golom	16	Southern Niger
Aguie	Laouni	16	Southern Niger
Aguie	Birnin Kouka	11	Southern Niger

For root nematodes, roots were washed, cut into pieces of 1 cm length and placed in a tripod sieve of 1 mm mesh. Each sieve was then deposited on a funnel extended by a pipe which end is closed by a lock-off. All sieves containing cut roots were placed in a moist chamber (Seinhorst, 1962a). After

one week, most of the second-stage juveniles were hatched and then were collected in tubes and counted.

### **Identification and frequency of plant parasitic nematodes**

The nematodes extracted from soil and roots were examined under a binocular microscope for the presence of other plant-parasitic nematodes. The nematode population densities in the roots were defined as the number of second-stage juveniles (J2) per gram (g) fresh roots and J2 per dm<sup>3</sup> of soil for *Meloidogyne* spp.

The incidence of root knot nematode infestation was determined by the following formula (Mohamud, 2014):

$$\text{Incidence} = \frac{\text{Number of samples showing nematode infestation}}{\text{Total number of samples examined}} \times 100$$

Root galling was assessed by examining the roots for the presence of galls. The root galling index used was based on the percentage of galled roots (Speijer & De Waele, 1997).

0 = no galling

1 = a few small galls

2 = < 25% of the roots galled

3 = 25–50% of the roots galled

4 = 50–75% of the roots galled

5 = > 75% of the roots galled

The importance of all plant-parasitic nematodes was determined from the diagram of frequency and abundance (Fortuner & Merny, 1973). For each genus, frequency and abundance were calculated as per the following:

The frequency (F) corresponds to the number (%) of locations where the species is found; a species occurring in more than 30 % of the locations is considered as frequent

$F = \frac{e}{n} * 100$  where e = total number of samples containing given genus/species of nematode and n = total number of samples at given site.

The abundance of a genus corresponds to log N, where N is the mean number, calculated on the samples where the species is present, of the individuals present in 1 dm<sup>3</sup> of soil, one species which is more numerous than 300 individuals per dm<sup>3</sup> of soil (log 300 = 2.47) or is considered as abundant.

Abundance (A) of nematode genus/specie is the average density per samples in which nematode was found. It was calculated using the following formula:  $A = \frac{\sum Xi}{e}$  where Xi = number of nematodes per liter of soil and e = number of samples in which the given nematode was present. According to Fortuner and Merny (1973) the diagram (Figure 7) is divided in 4 areas:

- Upper right area of the diagram, species frequent and abundant;
- Lower right area of the diagram, species frequent and not abundant;
- Upper left area of the diagram, species not frequent but abundant.
- Lower left area of the diagram, species not frequent and not abundant;

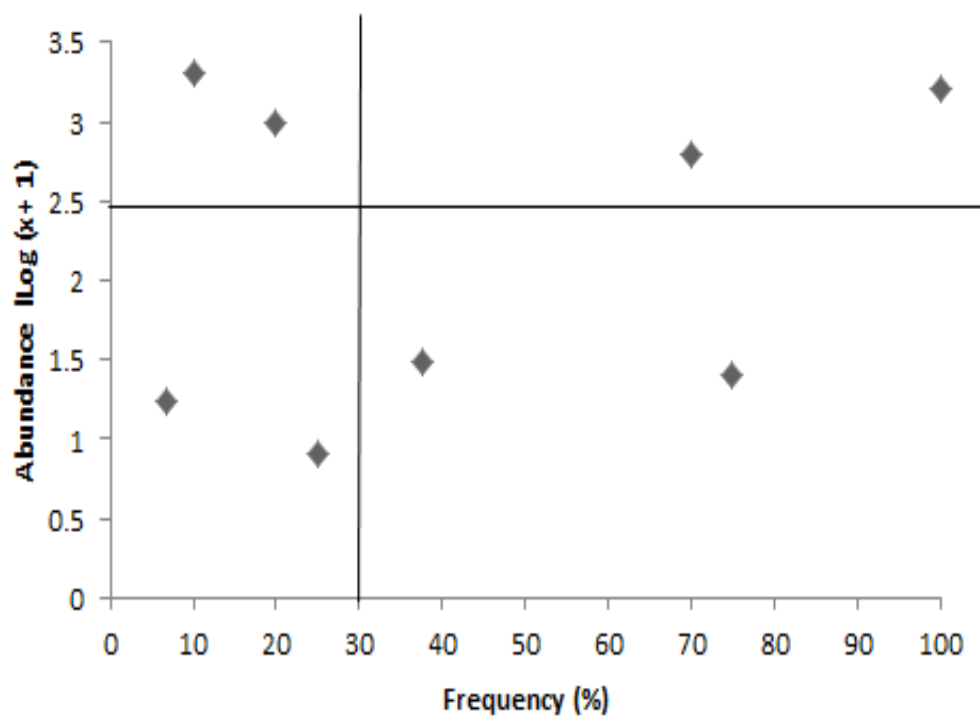


Figure 7: Frequency/Abundance of *Meloidogyne* species (Fortuner & Merny, 1973)

### Morphological and molecular characterization of *Meloidogyne* spp.

Morphological and molecular identification of *Meloidogyne* spp. and *Fusarium* spp. were conducted in the molecular phytomedicin Laboratory at INRES, University of Bonn, Germany. For *Meloidogyne* spp., seven isolates (populations) (Table 3), including 2 isolates from Aguié and 5 isolates from Diffa were collected from sweet pepper field survey throughout Diffa and Aguié.

Table 3-List of *Meloidogyne* populations used in species identification

Plant	District	Sampled site	Code number	Lab Code
Sweet pepper	Aguie	Gourgia	1	Gou2
Sweet pepper	Aguie	Gourgia	2	Gou2
Sweet pepper	Diffa	Boulangouri	3	Bou77
Sweet pepper	Diffa	Boulangouri	4	Bou76
Sweet pepper	Diffa	Fiego	5	Fie 52
Sweet pepper	Diffa	Fiego	6	Fie 58
Sweet pepper	Diffa	Bagara	7	Ba124

Second-stage juveniles (J2) obtained from a single egg mass isolates were reared and maintained on susceptible tomato plants (*Solanum lycopersicum* cv. Roma). The tomato plants were cultivated in a greenhouse in pots using soil autoclaved at 120°C for 4 hours.

#### **Morphological identification of *Meloidogyne* spp.**

For identification, mature females were collected from galled roots, while second-stage juveniles and males were extracted by blending roots in 1.5% NaCl solution for 3 min (Hussey & Barker, 1973).

#### ***Killing and fixing of second-stage juveniles (J2) and males***

Second-stage juveniles and males were killed and fixed according to Seinhorst (1962b). The following solutions were used to fix nematodes:

Formalin (40% formaldehyde)	10 ml
Glacial Acetic Acid (acid propionic)	1 ml
Distilled water	89 ml

Live J2s and males were picked up and transferred in a drop of water in staining blocks and properly labelled. The collected specimens were killed and fixed by adding 4% hot (60-80°C) formaldehyde + propionic acid to a small drop of water in a glass cavity vessel which contained the nematodes and processed glycerine by Seinhorst's method (Seinhorst, 1962b, 1966). Specimen preparation and measurements of the different characters like Body length, Stylet length, and Stylet base to head end, greatest width, Tail length, and Hyaline terminus for J2s were observed and recorded using the DMI2000 compound microscope (Leica Microsystems). For males, Body length, Stylet length, Knob width, Knob height, DGO, Body width, Width at knobs was measured.

***Preparation of perineal patterns of females***

The adult females of *Meloidogyne* spp. were removed from the root tissues by teasing apart with the help of fine forceps and were collected in lactic acid. The females collected were dissected and prepared for morphological identification based on the perineal pattern. The posterior end of females having vulva and anus was cut with a blade and was cleared in a solution of lactic acid to remove remaining body tissues. The perineal pattern was transferred in a drop of glycerol on a clean glass slide. A total of 42 perineal patterns were examined for species identification.

## **Molecular identification of *Meloidogyne* spp.**

### ***DNA extraction, PCR amplification and sequencing***

The total genomic DNA was extracted from individual J2s from seven populations using Quick-DNA Universal Kit from ZYMO RESEARCH Company, Germany following the manufacturer's instructions. The primers used for molecular identification of *Meloidogyne* species are presented in Table 4.

An aliquot of 5 µl of the suspension was used as a template for PCR reactions. To facilitate the identification, a standard genomic DNA of previously identified *M. incognita*, *M. javanica* and *M. arenaria* cultures (from Molecular Phytomedicin laboratory at INRES, Bonn, Germany) were extracted and used for comparison. For PCR, the published protocol to amplify the mtDNA region between the cytochrome oxidase subunit II (COII) using the primers C2F3/1108 (Powers & Harris, 1993) was followed. Furthermore, Me-F/Me-R specific for *M. enterolobii* were used (Long, Liu, & Xu, 2006). Polymerase chain reaction was performed in 25 µl reaction volume containing 5.5 µl of nuclease-free water (Sigma Aldrich® Company), 1X green Gotaq buffer (Promega), 0.5 mM dNTP mix (Promega), 1nM of each primer, 5ng of template DNA and 0.3 Unit Taq DNA Polymerase (Promega).

Table 4-Primers used for molecular identification of *Meloidogyne* species

Primer code	Primer sequence 5' -3'	Source
C2F3	GGTCAATGTTTCAGAAATTTGTGG	Powers and
1108	TACCTTTGACCAATCACGCT	Harris (1993)
Me-F	AACTTTTGTGAAAGTGCCGCTG	Long et al., 2006
Me-R	TCAGTTCAGGCAGGATCAACC	

### Electrophoresis and sequences analysis

DNA amplification products were separated on a 1% (w/v) agarose gel in 1×TAE (Tris-acetate-EDTA) buffer and PeqGreen as much as 4-5 µl / 100 ml agarose. Electrophoresis was performed at 80 volts for 60 minutes and visualized using UV light. The amplified DNA products were purified using NucleoSpin<sup>®</sup> Gel and PCR Clean-up Kit (MACHEREY-NAGEL, Düren, Germany) according to the manufacturer's protocol and checked for quality and concentration using the Nanodrop C2000 Spectrophotometer (Thermo Scientific, Passau, Germany). The purified PCR fragments from all 18 isolates were sequenced in both directions at GATC Biotech (Constanz, Germany).

DNA fragments from all 7 isolates were sequenced in both directions. DNA sequences were checked for quality and processed with CLC genomic workbench before blasting it in against the NBCI database.



## **Morphological and molecular characterization of *Fusarium* spp. isolated from sweet pepper**

### **Isolation and frequency of *Fusarium* spp.**

Stems of infected sweet pepper plants were washed to remove the adhering soil. The stems samples were cut into small pieces up to 1.5 cm length and surface sterilized with 1% of NaClO for 3 minutes, rinsed with distilled water three times and then air-dried. The surface sterilized tissues were placed on Malachite Green Agar (MGA) medium (Castella, Bragulat, & Cabanes, 1997) and incubated at 25°C. After 6 days the fungal isolates appearing on the stem pieces were subcultured unto 2% Malt Extract Agar (MEA) medium Petri plates for purification. Single spore of the pure culture was plated on Potato Dextrose Agar (PDA) medium, incubated at 27°C, and stored at 10°C until use.

### **Morphological characterization of *Fusarium* spp.**

For *Fusarium* spp., 18 isolates were randomly selected including 8 isolates from Aguié and 10 isolates from Diffa collected from sweet pepper fields (Table 5). Morphological identification of *Fusarium* spp. was based on characteristics of single spore isolates described by Leslie and Summerell (2006). The isolates were cultured on Carnation Leaf Agar (CLA) medium at 25°C near-ultraviolet light for 2 weeks (Fisher et al., 1982). The macroconidia, microconidia, phialides, and other features were then examined under DMI2000 compound microscope (Leica Microsystems). Twenty macroconidia were observed randomly, and the width and length were measured.

Table 5-List of *Fusarium* isolates used in species identification

Plant	District	sampling site	code number	lab code
Sweet pepper	Aguie	Gourgia	1	Gou 2
Sweet pepper	Aguie	Gourgia	2	Gou 10
Sweet pepper	Aguie	Gourgia	3	Gou15
Sweet pepper	Aguie	Gourgia	4	Gou16
Sweet pepper	Aguie	Assaya	5	Assa26
Sweet pepper	Aguie	Assaya	6	Assa26
Sweet pepper	Aguie	Glom	7	Glo58
Sweet pepper	Aguie	Glom	8	Glo57
Sweet pepper	Diffa	Fiego	9	Fie 52
Sweet pepper	Diffa	Fiego	10	Fie 58
Sweet pepper	Diffa	Boulangouri	11	Bou76
Sweet pepper	Diffa	Boulangouri	12	Bou77
Sweet pepper	Diffa	Boulangouri	13	Bou78
Sweet pepper	Diffa	Boulangouri	14	Bou80
Sweet pepper	Diffa	Ngarwa	15	Ngar29
Sweet pepper	Diffa	Ngarwa	16	Ngar31
Sweet pepper	Diffa	Ngarwa Gana	17	Gan29
Sweet pepper	Diffa	Ngarwa Gana	18	Gan35

## **Molecular identification of *Fusarium* spp.**

### **DNA extraction and PCR amplification**

*Fusarium* isolates were grown on 2% MEA (Castella et al., 1997) for 10 days at 27°C. Mycelia was harvested and used immediately for DNA extraction. The total genomic DNA was extracted from the fungal isolates using Quick-DNA Universal Kit ZYMO RESEARCH Company, Germany following the manufacturer's instructions. The primers used for molecular identification of *Fusarium* isolates are presented in Table 6.

For accurate identification of the isolates, universal primers ITS1/ITS4 primers (White, Bruns, Lee, Taylor, 1990) coupled with translation elongation factor (EF-1 $\alpha$ ) primers EF1/EF2 (O'Donnel et al., 1998) were used. PCR amplification was conducted in a 50  $\mu$ l reaction mixture containing 4  $\mu$ l of fungal DNA, 1  $\mu$ l of each primer (10 mM), 1  $\mu$ l dNTPs mix (10 mM ) (Promega), ( 1.5 Unit ) of Taq DNA Polymerase (Promega), 10  $\mu$ l reaction buffer (5x Green Go Taq® reaction buffer (Promega) and 32.5  $\mu$ l of nuclease-free water (Sigma Aldrich® Company).

For ITS amplification, the PCR was performed by using the following amplification cycles: initial denaturation at 95°C for 4 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 2 min, and elongation at 72°C for 3 min, and followed by a final extension of 72°C for 10 min. For TEF-1 $\alpha$  amplification, the PCR conditions were according to Nitschke et al., (2009). The electrophoresis and sequences analysis were done as in section (**Electrophoresis and sequences analysis**).

Table 6-Primers used for molecular identification of *Fusarium* isolates

Primer code	Primer sequence 5' -3'	Source
ITS1	TCCGTAGGTGAACCTGCGG	White et al., 1990
ITS4	TCCTCCGCTTATTGATATGC	
EF1	ATGGGTAAGGA(A/G)GACAAGAC	O'Donnel et al.,
EF2	GGA(G/A)GTACCAGT(G/C)ATCATGTT	1998

#### **Pathogenicity test with *Fusarium* isolates**

Pathogenicity test of the *Fusarium* isolates were done on plants and mature fruits of sweet pepper. Due to the identical morphological and molecular characteristic of the isolates, eight of them were selected randomly for the inoculation of the host susceptible local sweet pepper (crotte d'âne) in two fruits or plants per isolate

The test was carried out on mature green coloured fruits of sweet pepper. The conidia suspension for inoculation was prepared from fungal cultures grown on PDA, by harvesting the mycelium, scraping the sporulating colonies, suspending in sterile distilled water, and shaking vigorously. The conidial suspensions were transferred to sterile tubes then filtered to remove hyphae by using a 45 µm sieve. The conidial concentration was adjusted to 10<sup>6</sup> spores/ml using a hemacytometer (Neubauer) and microscope (Petrikkou et al., 2000). The fruits were wounded and inoculated with the conidial suspension using a sterile brush. Control treatment was inoculated with distilled water. The inoculated fruits were incubated at 25-28°C for two weeks

in a humid chamber at 80% RH. Changes in colour and softness in the fruit tissues were recorded after the first week of incubation.

### **Effect of temperature on co-infection of *Meloidogyne enterolobii* and *Fusarium solani* on the severity of wilt of sweet pepper plants in Niger**

The experiment was carried out in a growth chamber (Sanyo versatile environmental test chamber, MLR-351H) at the AGRHYMET regional centre, Niamey (Niger).

#### **Host plant**

A local variety of sweet pepper called “*kangadi-n 'glaro*” from Diffa district was used in this experiment. Seeds were surfaced sterilized with 1% of NaClO for 3 minutes and washed with distilled water before sowing. After germination, at the stage of five to six leaves, three (3) plants were transferred to plastic pots (15cm-diameter) filled with a mixture of sterilized sandy soil collected from sweet pepper growing area in Diffa.

#### **Preparation of nematode inoculum**

A pure culture of *M. enterolobii* isolated from sweet pepper fields using a single egg mass technique and maintained on tomato roots in pots in the greenhouse was used for this study. To obtain the nematode inoculum, heavily infected roots were washed and nematodes were extracted from roots as described by Seinhorst (1962a). Freshly hatched second stage juveniles (J2) 2000 J2/pot were used for inoculation.

#### **Preparation of fungal inoculum**

A pure culture of *F. solani* isolated from sweet pepper roots and maintained on Potato Dextrose Agar (PDA) was used for this study. The

*Fusarium* inoculum was prepared as follows: mycelium collected from (10- to 14-day-old) colonies were harvested by scraping the sporulating colonies and suspending in sterile distilled water, shaken vigorously. The conidial suspensions was transferred to a sterile tube then filtered to remove hyphae using a 45 µm sieve 45µm. Conidia concentration was then adjusted to 10<sup>6</sup> spores/ml using microscopic enumeration with a cell-counting hemacytometer Neubauer chamber (Petrikkou et al., 2000) and used for inoculation.

### **Inoculation method and Experimental design**

Experiments were conducted three times in a growth chamber. For inoculation of the plants, a hole was made in the soil close to the stem base of each plant. Sweet pepper plants in pots were inoculated with (2000 J2/pot) of *M. enterolobii* and *F. solani* (20 ml 10<sup>6</sup> spores/pot). The isolate of *F. solani* used was collected from sweet pepper and it proved its ability to colonize fruits of sweet pepper. Inoculation of *Meloidogyne* and *Fusarium* was accomplished by pipetting the appropriate concentration of second stage juveniles and spores onto each sweet pepper plant and immediately covering with sterilized soil. The following treatments were assessed:

1. Plants inoculated with *M. enterolobii* alone (Me)
2. Plants inoculated with *F. solani* alone (Fs)
3. Plants inoculated with *M. enterolobii*, after 1 week followed by an inoculation with *F. solani* (MeFs)
4. Plants inoculated with *F. solani*, after 1 week followed by an inoculation with *M. enterolobii* (FsMe)

5. Plants inoculated with *M. enterolobii* and *F. solani* simultaneously (Me + Fs)
6. Control plants: no *Meloidogyne*, no *Fusarium* (nMenFs)

The inoculated and control plants were placed in a growth chamber in complete randomized design (CRD) with six treatments and four replications and grown at three different temperatures (T1=23°C, T2= 27°C and T3= 30°C). Throughout the experiment the plants were lightened by incandescent lamps with a photoperiod of 12 hours. The watering was done necessary.

Eight weeks after inoculation, plants were harvested for evaluation of total fresh biomass and root weight, plants height, root length, yellow leaves, and number of galls. The number of J2/500 g soil, J2/ g roots and J2/root system were counted after extraction by Seinhorst method (Seinhorst 1962a). In addition, to evaluate the *Fusarium* incidence, the plant stems were sterilized with 1% of NaClO, washed with distilled water, and then air-dried. the dried stems were then cut into small pieces up to 0.5 cm length and surface sterilized by 0.525% NaClO to eliminate eventual contamination, then rinsed with distilled water three times. The sterilized stems were placed onto 2% MEA (Castella et al., 1997) and incubated for one week.

#### **Statistical analysis of data**

All statistical analyses were performed using R Core Team (2015). R: A language and environment for statistical computing R 64 3.3.0. The one way ANOVA was used to test significance of treatment on main variables response. Tukey honestly significant difference (HSD) test at  $P < 0.05$  (with the Tuckey HDS function from R) was used as *post-hoc* to test whether the

variance of mean was greater between treatment within single main variable. The significance of predictors on response variable was tested using an analysis of variance (ANOVA) with type III sum of squares. This was done using the "lm" and the "drop1" functions from R.

### **Perception of farmers on climate variability, adaptation strategies and access to agricultural extension services in Diffa and Aguié**

#### **Data collection**

The study used both quantitative and qualitative information. Twenty years daily meteorological data of temperature and rainfall of Diffa and Aguié districts obtained from the AGRHYMET Regional Centre were analysed. The multiple-stage and simple random sampling procedure were used to select a sample of 284 and 98 respondents respectively from the Diffa and Aguié districts. The target population for this study were farmers in Diffa and Aguié districts involved in rainfed and irrigated agriculture. A structured questionnaire with closed and open-ended questions was administered to collect information on perceptions of changes in rainfall amount, temperature, onset and end of rainy season, length of the growing season, access to extensions services and adaptations over the last 20 years. Data were collected from 2013 and 2014 by face-to-face interviews with the farmers.

#### **Data analysis**

Instat+ software, version 3.36 was used for statistical analysis of daily rainfall and temperature data to estimate the onset and end of the rainy season and the length of growing period. Statistical Package for Social Sciences



(SPSS) 20th edition was used for basic descriptive statistical analyses. Means, percentages and frequencies were used to summarize and categorize the information gathered from farmers. The perception of farmers on weather patterns were compared to meteorological observations. For linear trend, T-test ( $p = 0.05$ ) was run on annual means of climatic parameters to check significance.

In this study the phrase “Crop diversification” refers to mixed cropping that is aimed to increase crop portfolio so that farmers are not dependent on a single crop to generate their income. Planting trees refers to a strategy that farmers implement to prevent violent winds or other disasters in order to protect their crops. To investigate the adaptation strategies in depth, further analysis on sub-group basis were done: farmers with less than 20 years of farming experience and those with more than 20 years.

## CHAPTER FOUR

### RESULTS

#### **Distribution, frequency of occurrence and importance of plant-parasitic nematode associated with sweet pepper**

##### **Disease symptoms**

During the survey 199 soil and root samples were collected from sweet pepper plants in two districts Diffa and Aguié in Niger. The symptoms of infected sweet pepper plants observed in the fields were yellowing, stunted growth, plant death and heavily galled and rotted root systems (Figure 8 A-D).

##### **Incidence, gall index and J2 population of *Meloidogyne* spp. on sweet pepper in Diffa and Aguié**

The overall incidence (Figure 9) was 100% at all locations in both districts in both districts. Gall index significantly varied within the sampling sites on direct examination of plant root system using 0-5 scale. The overall gall index ranged from 1 to 5. While galling index ranged from 1 to 3 in Diffa, it ranged from 4 to 5 in Aguié (Figure 10). The population density of *Meloidogyne* spp. also significantly varied in Diffa and Aguié. The overall population density of *Meloidogyne* spp. ranges from 599 to 11548/g root (Figure 11). In Diffa, *Meloidogyne* spp population in root and soil was 599-1548 and 663-1757 respectively, while *Meloidogyne* density was 1758-4556 and 364-932 in Aguié.



Figure 8: Sweet pepper plants infected by *Meloidogyne* spp: A healthy plants, B: infected sweet pepper plants with yellowing and wilting symptoms. (C-D) Sweet pepper roots with severe galling.

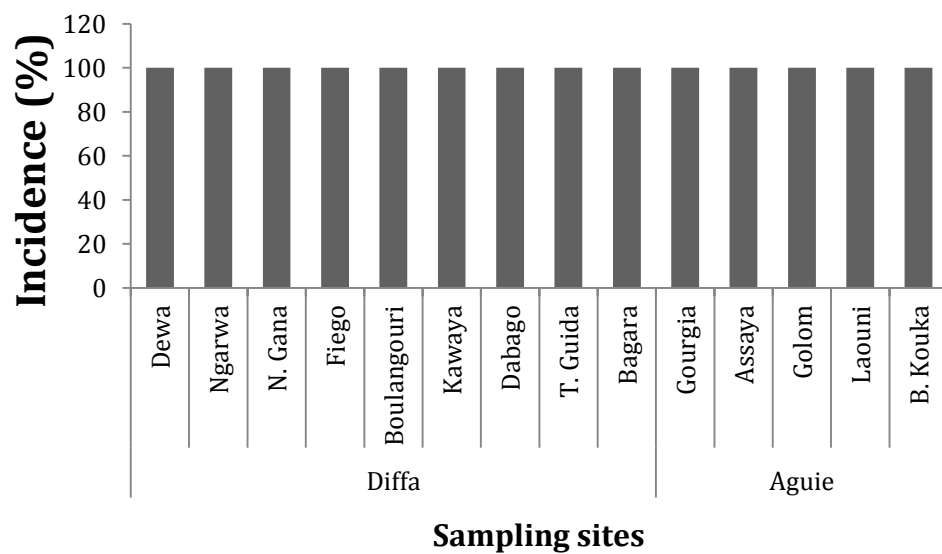


Figure 9: Incidence of *Meloidogyne* spp. on sweet pepper in Diffa and Aguié

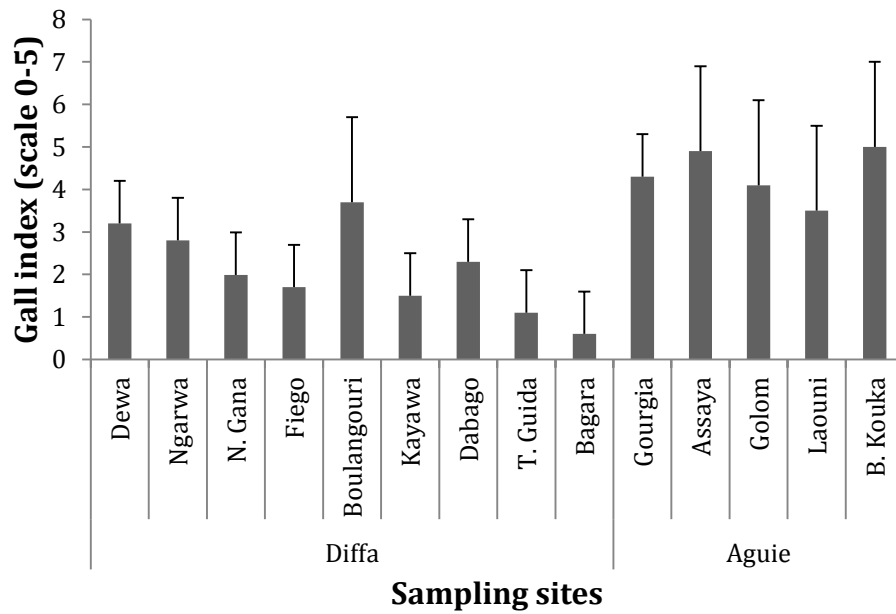


Figure 10: Gall index of *Meloidogyne* spp. on sweet pepper in Diffa and Aguié

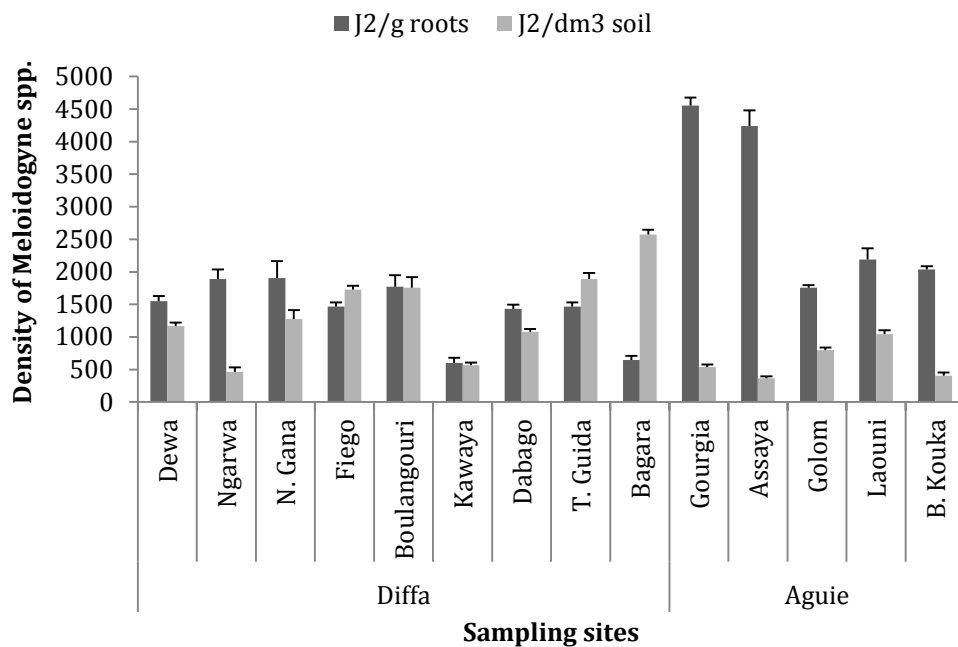


Figure 11: Population density of *Meloidogyne* spp. on sweet pepper in Diffa and Aguié

## Distribution, frequency of occurrence and importance of plant-parasitic nematodes

The generic distribution of plant parasitic nematodes at all 14 sampled sites is given in Figure 12. In this study ten (10) genera of parasitic nematodes, *Meloidogyne*, *Tylenchorynchus*, *Helicotylenchus*, *Criconemella*, *Xiphinema*, *Scutellonema*, *Hoplolaimus*, *Rotylenchulus*, *Pratylenchus* and *Trichodorus* were found. Out of the 10, *Meloidogyne* were present at all sites surveyed. *Tylenchorynchus* and *Helicotylenchus* were found in almost all sites except in Bagara where *Helicotylenchus* was not present and, in Birnin Kouka where *Tylenchorynchus* was absent. Some plant-parasitic nematode species occurred only in one agroecological region.

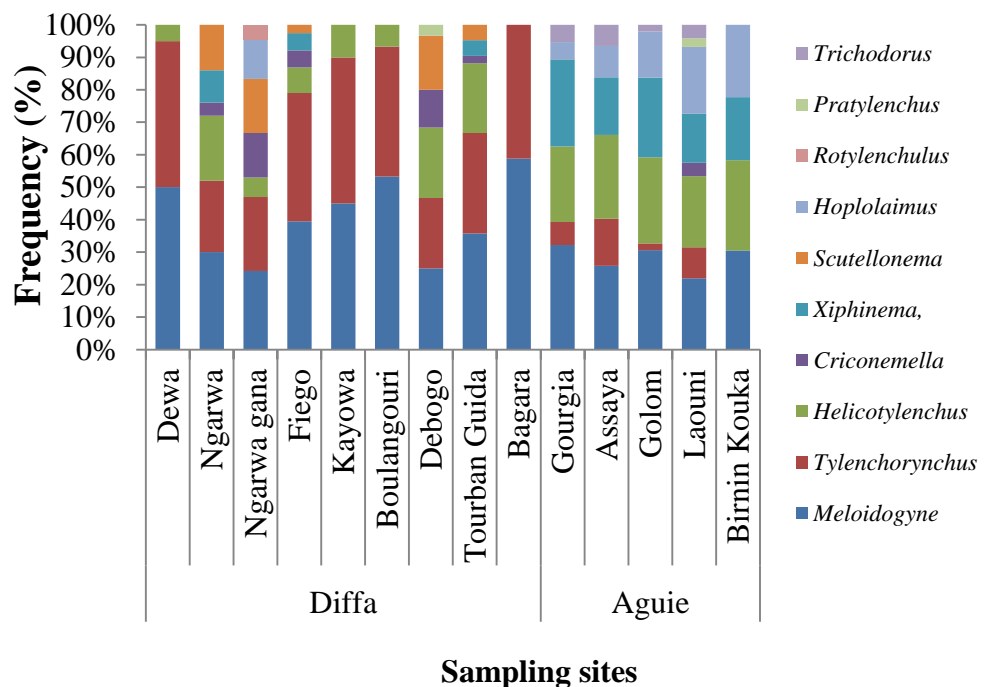


Figure 12: Distribution and frequency of plant parasitic nematodes in Diffa and Aguié

The importance group of plant parasitic nematodes is given in Figures 28 and 29 respectively in Diffa and Aguié.

Three groups of species can be distinguished according to their global frequency and abundance in soil in Diffa (Figure 13):

The first, being those that occurred frequently and abundantly, (*Meloidogyne*, *Helicotylenchus*, *Tylenchorynchus*), and could be located in the Upper right area of the diagram. The second group were those situated in the Upper left area of the diagram which were not frequent but abundant (*Scutellonema*) and the third situated in Lower left area of diagram that were not frequent and not abundant (*Criconemella*, *Xiphinema*, *Hoplolaimus*, *Rotylenchulus*, *Pratylenchus*, *Trichodorus*).

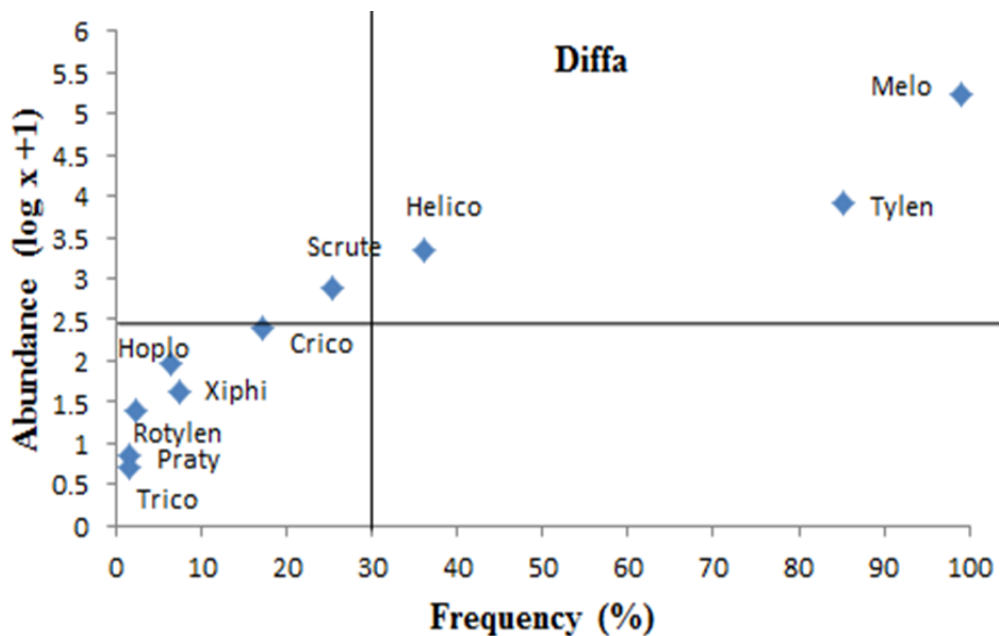


Figure 13: Importance of plant parasitic nematodes in Diffa. Scute = *Scutellonema*, Crico = *Criconemella*, Xiphi = *Xiphinema*, Hoplo = *Hoplolaimus*, Rotylen = *Rotylenchulus*, Praty = *Pratylenchus*, Trico = *Trichodorus*, Melo = *Meloidogyne*, Helico = *Helicotylenchus*, Tylen = *Tylenchorynchus*.

In contrast, two groups of species could be distinguished according to their global frequency and abundance in soil in Aguie (Figure 14). The first group, (points situated in the upper right area of the diagram), occurred very frequently and were abundant: *Meloidogyne*, *Helicotylenchus*, *Xiphinema*, and *Hoplolaimus*. Nematodes of the second group (points situated in Lower left area of the diagram), are species that were not frequently found and not abundant: *Tylenchorynchus*, *Trichodorus*, *Criconemella*, *Pratylenchus*.

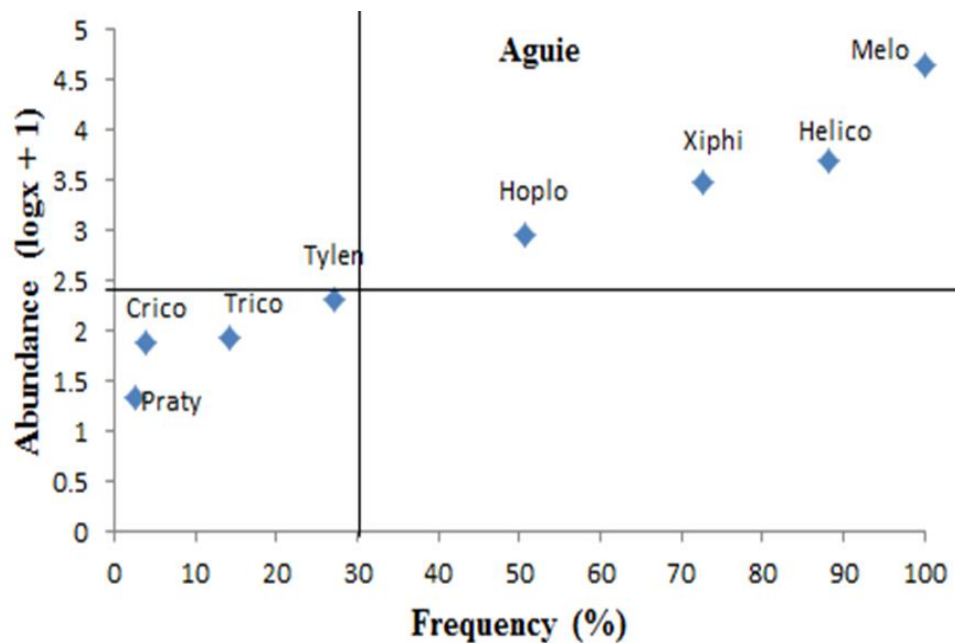
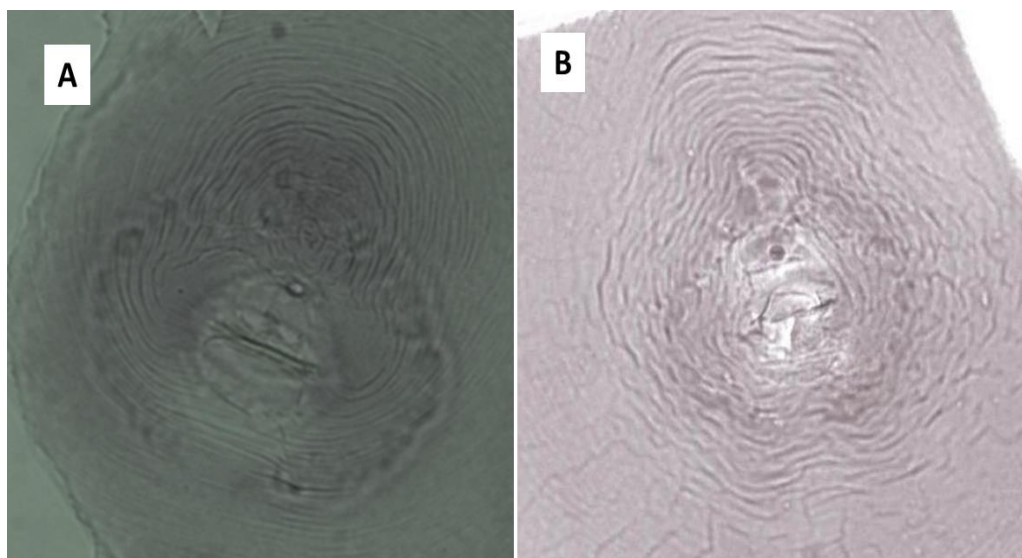


Figure 14: Importance of plant parasitic nematodes in Aguie. Melo = *Meloidogyne*, Helico = *Helicotylenchus*, Tylen = *Tylenchorynchus*, Trico = *Trichodorus*, Praty = *Pratylenchus*, Crico = *Criconemella*, Xiphi = *Xiphinema*, Hoplo = *Hoplolaimus*.

**Morphological characterization of *Meloidogyne* spp. with emphasis on *M. enterolobii***

**Morphological observations of perineal patterns**

The perineal patterns of females (population from isolates (1-6) were oval shaped with moderate to high dorso-ventrally ovoid perineal pattern (Figure 15 A). In some cases, those perineal patterns show high dorsal arches round or oval and with or without lateral lines (Figure 16 (F-J)) which are similar to *M. enterolobii*. The perineal patterns of females from isolate 7 (Figure 15B) were characterized by a high trapezoidal dorsal arch and narrow dorsal curve and similar to that of *M. incognita*.



*Figure 15: Photomicrographs of perineal patterns: (A) Meloidogyne enterolobii, (B) Meloidogyne incognita. Scale bar for all figures = 75  $\mu$ m*



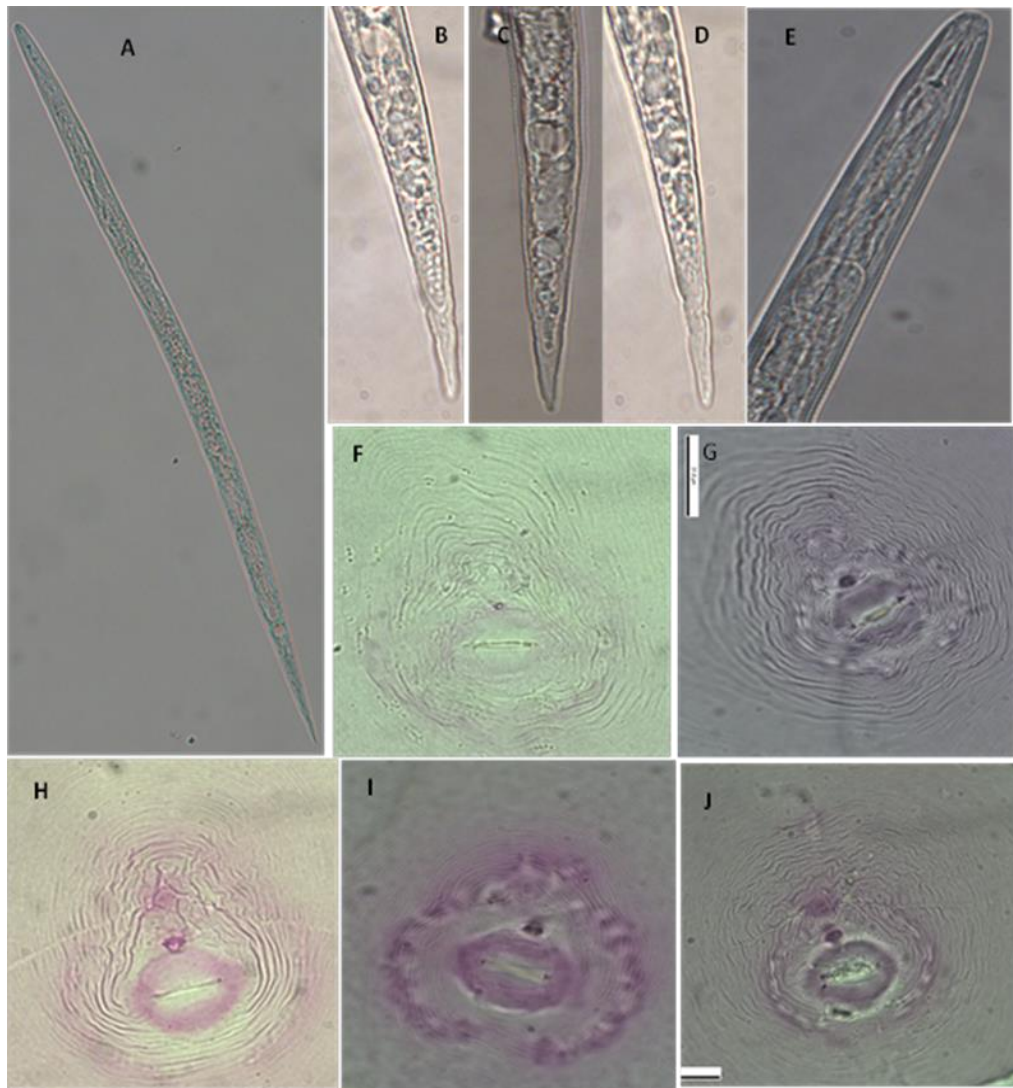


Figure 16: Photomicrographs of *Meloidogyne enterolobii* second-stage juveniles and perineal patterns of females: A: whole body of second stage, B to D: tails. E: head of second stage, F to J: variability of perineal patterns. Scale bar for all figures = 75  $\mu$ m.

### Morphometric measurements

Morphometrics of the J2s and males from all six isolates of *M. enterolobii* are listed in Tables 8 and 9 respectively. The six isolates were morphologically similar to each other when compared to the original description of *M. enterolobii* (Yang & Eisenback, 1983). The mean body

length of J2s of six isolates ranged from 395 to 410  $\mu\text{m}$  with the maximum body length recorded in isolate 2 (Table 8). The tail length average ranged from 50 to 56 $\mu\text{m}$  with the maximum tail length recorded in isolate 5 (Table 8).

The mean body-length of males ranged from 1331 to 1802  $\mu\text{m}$  and the average stylet-length from 21 to 23 $\mu\text{m}$  (Table 9). The Dorsal pharyngeal Gland Orifice (DGO) ranged from 3.4 to 4.7 $\mu\text{m}$  and mean body width range from 31 to 43  $\mu\text{m}$ . The mean body length of J2s of isolate 7 was between 349.4 to 398.2  $\mu\text{m}$  and the tail length average from 50 to 56  $\mu\text{m}$ . Morphometrics features of isolate 7 (Table 7) were compared to those reported by Brito et al., (2004).

Table 7-*Morphometrics (mean, standard deviation, and range) of second-stage juveniles of six isolates of Meloidogyne incognita from Niger*

Characters	Isolate 7 under study n = 22	<i>M. incognita</i> from Florida. Race 2 n = 20
Body length	373.1 $\pm$ 13.7 (349.4 - 398.2)	388.5 $\pm$ 18.8 (348.5–424.0)
Stylet length	11.8 $\pm$ 0.3 (11.1 - 12.7)	10.7 $\pm$ 0.3 (10.2–11.2)
Stylet base to head end	14.6 $\pm$ 1.1 (12.4 - 16.8)	14.9 $\pm$ 0.3 (14.5–15.6)
Greatest width	14.1 $\pm$ 0.9 (13.1 - 17.8)	14.7 $\pm$ 0.4 (14.2–16.0)
Tail length	56.9 $\pm$ 4.3 (52.3 -58.6)	49.6 $\pm$ 3.3 (44.1–52.9)
Hyaline terminus length	11.5 $\pm$ 1.0 (9.7 - 13.7)	11.5 $\pm$ 1.7 (9.7–13.7)

Table 8-Morphometrics (mean, standard deviation, and range) of second-stage juveniles of six isolates of *Meloidogyne enterolobii*

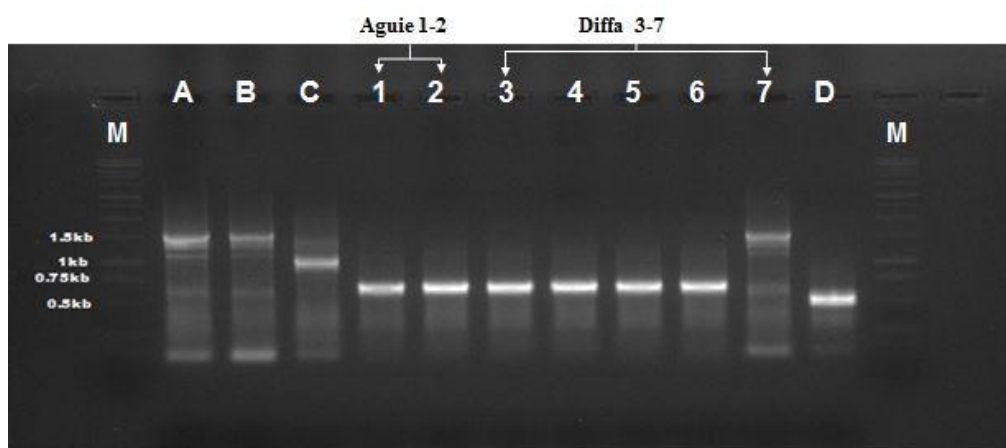
Second-stages Juveniles Characters	Aguie isolates (1-2)		Diffa isolates (3-6)				Original description(*)
	Isolate 1 n = 20	Isolate 2 n = 20	Isolate 3 n =24	Isolate 4 n = 25	Isolate 5 n = 25	Isolate 6 n = 21	
Body length	395.0 ± 17.4 (361.5 - 422.7)	410.2 ± 12.8 (384.7 ± 436.9)	401.4 ± 18.7 (371.4 - 446.7)	400.6 ± 15.1 (375.0 - 426.5)	409.5 ± 20.4 (372.0 - 459.7)	379.8 ± 31.3 (318.5 - 445.9)	436.6 ± 16.6 (405.0-472.9)
Stylet length	12.7 ± 0.4 (11.8 - 13.4)	11.9 ± 0.3 11.1 ± 12.5	11.9 ± 0.4 (10.9 - 12.8)	12.4 ± 0.4 (11.7 - 13.3)	12.0 ± 0.5 (10.7 - 13.1)	11.7 ± 0.6 (10.8 -13.2)	11.7 ± 0.45 (10.8-13.0)
Stylet base to head end	14.5 ± 0.2 (13.9 - 15.1)	14.3 ± 0.3 13.7 ± 14.6	14.3 ± 0.2 (13.7 - 14.7)	14.4 ± 0.3 (13.9 - 15.3)	14.6 ± 0.5 (13.4 - 15.4)	14.2 ± 0.6 (13.1 - 16.1)	--
Greatest width	15.8 ± 1.1 (14.1 - 18.5)	17.6 ± 0.7 16.1 ± 18.9	15.3 ± 1.2 (13.3 - 17.6)	16.0 ± 0.7 (14.7 - 18.1)	16.4 ± 0.6 (15.0 ± 17.8)	15.2 ± 0.8 (13.3 - 16.5)	15.3 ± 0.8 (13.9-17.8)
Tail length	55.6 ± 5.2 (43.6 - 62.9)	50.9 ± 6.0 42.9 ± 58.5	52.4 ± 5.6 (31.8 - 60.4)	52.1 ± 3.2 (47.2- 54.5)	56.9 ± 2.2 (53.4 - 61.3)	52.6 ± 5.9 (36.0 - 61.7)	56.4 ± 4.4 (41.5--63.4)
Hyaline terminus	12.3 ± 1.3 (8.9 - 14.4)	12.6 ± 1.3 10.1 ± 14.2	12.5 ± 1.7 (8.62 - 15.9)	12.7 ± 0.8 (11.4 - 14.1)	12.1 ± 1.4 (8.3 - 13.9)	11.1 ± 1.0 (9.5 -13.5)	--

Table 9-Morphometrics (mean, standard deviation, and range) of males of six isolates of *Meloidogyne enterolobii* from Niger

Males Characters	Aguie isolates (1-2)		Diffa isolates (3-6)				Original Description*
	Isolate 1 n =10	Isolate 2 n = 10	Isolate 3 n = 8	Isolate 4 n = 9	Isolate 5 n = 11	Isolate 6 n = 10	
Body length	1620.6 ± 256.0 (1124.4 - 2003.7)	1802.0 ± 217.5 (1391.3 - 1998.6)	1428.3±301.3 (1040.7- 1955.8)	1331.0±300 (984.3- 1909.6)	1697.5 ± 288.3 (953.6 - 1972.7)	1797.5 ± 288.3 (989.6 - 1972.7	1599.8 ± 159.9 (1,348.6-1,913.3 )
Stylet length	21.4 ± 1.0 (19.8 - 23.9)	21.3 ± 1.02 (19.4 - 22.6)	21.1±1 (20.1-22.6)	21.7±0.7 (20.5 - 23)	23.1 ± 0.74 (22.3 - 24.5)	21.1 ± 0.74 (21.3 - 23.5)	23.4 ± 0.9 (21.2-25.5 )
Knob width	5.0 ± 0.6 (4.3 - 6.3)	5.4 ± 0.4 (4.5 - 6.0)	4.7±0.4 4.0-5.3	4.8±0.3 4.5-5.1	4.8 ± 0.5 (4.4 - 5.8)	4.1 ± 0.5 (3.6 - 5.1)	5.4 ± 0.3 (4.5-5.8)
Knob height	2.9 ± 0.4 (2.3 - 4.0)	2.7 ± 0.2 (2.3 - 3.1)	2.6±0.3 2.2-2.9	2.6±0.2 2.1-2.9	2.5 ± 0.2 (2.2 - 3.0)	2.8 ± 0.2 (2.6 - 3.5)	3.3 ± 0.3 (2.6-3.9 )
DGO	4.5 ± 0.2 (4.3 - 5.0)	4.7 ± 0.4 (4.4 - 5.3)	4.8±0.5 (4.3-5.2)	4.2±0.8 (3.5-5.1)	4.4 ± 0.6 (2.8 - 4.2)	4.4 ± 0.6 (3.8 - 5.2)	4.7 ± 0.4 (3.7-5.3 )
Body width	37.2 ± 6.1 (25.0 - 44.9)	38.7 ± 4.0 (30.3 - 43.9)	31.5±4.8 24.7-38.5	33.8±5.7 26.3-43.4	43.5 ± 0.7 (43.0 - 44.0)	42.5 ± 0.7 (38.0 - 43.2)	42.3 ± 3.5 (37.0-48.3 )
Width at knobs	18.7 ± 1.1 (16.5 - 20.7)	19.8 ± 1.5 (16.1 - 22.2)	17.8±1.3 15.9-19.3	18.0±1.1 16.4-18.8	21.2 ± 1.44 (19.4 - 22.6)	20.2 ± 1.44 (18.8 - 21.6)	--

### Molecular characterization of *Meloidogyne* spp.

The primer set C2F3 and 1108 was used to amplify the COII/16S rRNA gene of the mitochondrial DNA (Powers & Harris 1983). Amplification of DNA extracted from J2s with C2F3/1108 (Figure 17) yielded 1700~bp in lanes A and B respectively for *M. incognita* and *M. javanica*, in lane C and D produced 1100 and 600-bp respectively for *M. arenaria* and *M. graminicola*. Its amplification produced single amplicon of 705~bp from each of the six isolates (1-6), while for isolate 7, PCR product was approximately 1.6 kb.



*Figure 17:* Gel image of C2F3/1108 amplified PCR product. The 1.7 kb sizes of PCR products are characteristics of A = *M. incognita* and B = *M. javanica* 1.1kb characteristics of C = *M. arenaria* ~600bp in lane D = *M. graminicola*, lanes 1-6 produced approximately about 705-bp characteristics of *M. enterolobii*. Lane 7 produced 1.6 kb as *M. incognita*, M = lanes were loaded with 1 kb DNA-Ladder (Promega)

Additional primers set specific to *Meloidogyne enterolobii*, Me-F/Me-R (Long et al. 2006) were used to amplify rDNA-IGS2 sequences of six populations of *Meloidogyne enterolobii*. PCR amplification produced single bright amplicon of 200 bp (Figure 18) whereas no product was amplified from the others nematodes. The *Hinf I* digestion of the 1.6kb (Figure 19) determined

single digestion site generating two fragments of 1.2 and 0.37 kb. Furthermore, the sequences of isolate 7 were compared to the *M. incognita* (accession number KF993635 from genbank database). The result (Figure 20) is based on single digestion site for *Hinf I*.

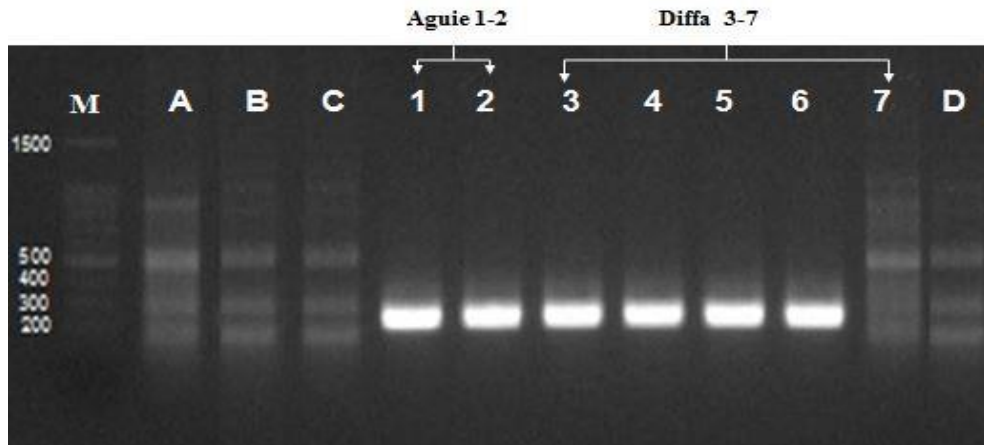


Figure 18: Gel image of Me-F / Me-R amplified PCR product. The 200-bp sizes of PCR products are characteristics of *M. enterolobii*. M= lanes were loaded with 1kb DNA-Ladder (Promega).

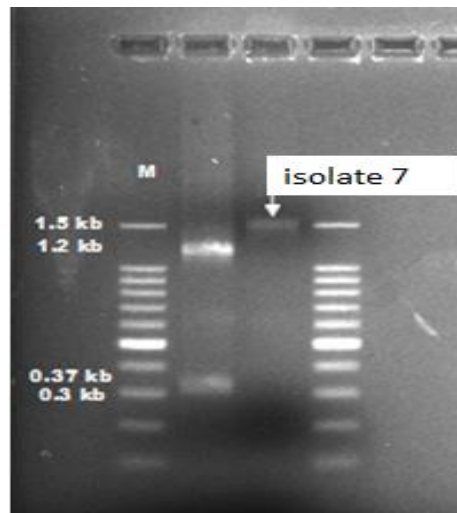


Figure 19: Gel image of PCR products from isolate 7 restricted with *Hinf-I* enzyme. Lane showing two fragments of 1.2 and 0.37 kb were related *M. incognita*. The products were resolved on 1.2% agarose gel. M lanes were loaded with 100-bp ladder (Promega)

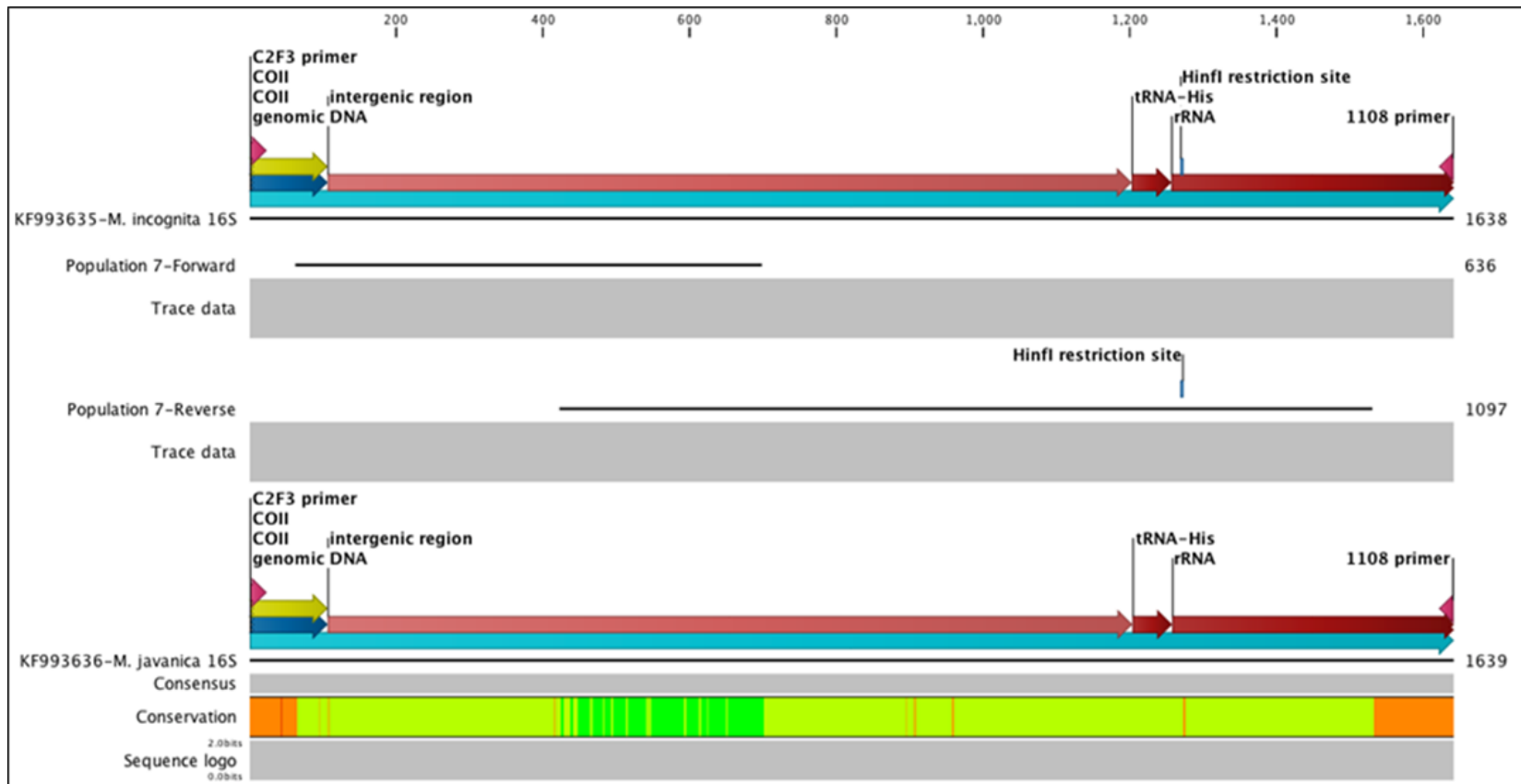


Figure 20: Hinf-I restriction enzyme indicating on single digestion on isolates 7 (population 7)

## Frequency of occurrence *Fusarium* spp. associated with sweet pepper

### Disease symptoms

During the survey, the symptoms of *Fusarium* infected sweet pepper plants in the fields were yellowing and wilting in some case (Figure 21) and fruit rot on other fields during the harvest (Figure 22).



Figure 21: Wilted sweet pepper plants on fields



Figure 22: Rotten fruits of sweet pepper harvested from the fields



## Morphological and molecular characterization of *Fusarium* spp.

### Isolation and frequency of *Fusarium* spp.

The same root and stem samples used for nematode extraction were examined for *Fusarium* occurrence. A total of 199 were examined. Based on the colour specifications of colony of isolates and microscopic characteristics, *Fusarium* spp. were found in 98 samples from Diffa and in 48 samples from Aguié. The *Fusarium* frequency was 80.32% in Diffa and 62.23% in Aguié (Figure 23).

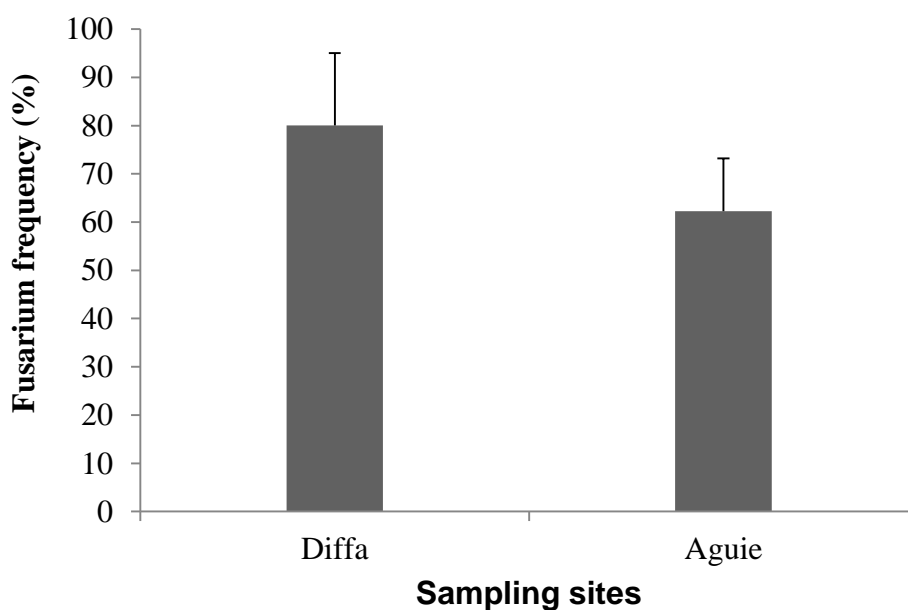
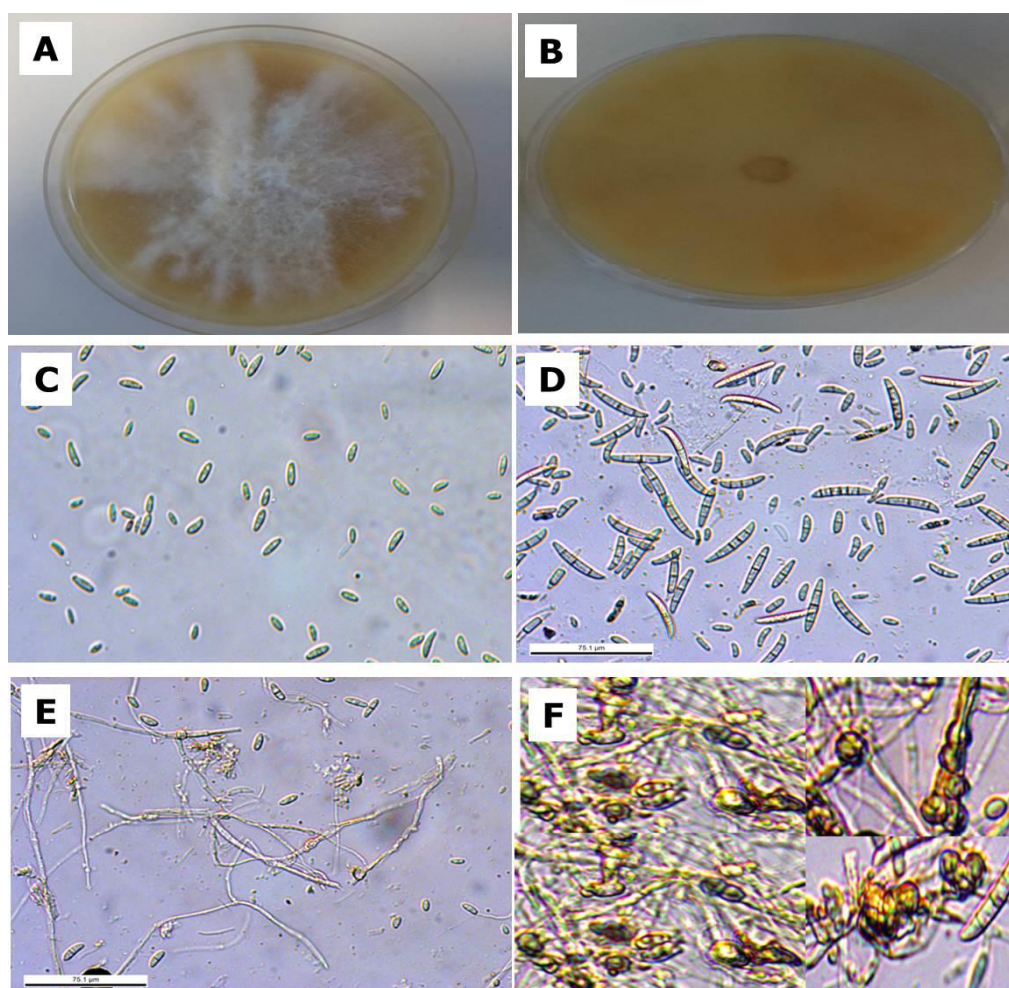


Figure 23: *Fusarium* frequency on sweet pepper in Diffa and Aguié

### Morphological characterization of *Fusarium* spp.

The 18 isolates of *Fusarium* spp. collected from Diffa (10) and Aguié (8) are similar in appearance on PDA. The fungi produced white aerial mycelia, cream to yellowish colonies on PDA (Figure 24A). The microscopic characteristics of isolates on carnation leaf agar (CLA) are presented in Figure

24(C-F). The isolates produced abundant microconidia and macroconidia. The conidiophores were unbranched and monophialides and the microconidia were usually aseptate or single septate, oval-ellipsoidal in shape and  $11\sim15 \times 2.5\sim4$   $\mu\text{m}$  sizes. Macroconidia were sparse, straight to slightly curved measuring  $36\sim48 \times 4.5\sim6$   $\mu\text{m}$  sizes with slightly curve or slender shape having 3~6 septates. Sporodochia were rare on CLA.



*Figure 24:* Morphological characters of *Fusarium solani* isolated from sweet pepper. The fungal isolate was grown on carnation leaf agar CLA for microscopic observation. Colony shape: A= front and B = back side of PDA plate. C = microconidia, D= macroconidia, E = = microconidiophores and F= chlamydospores formed on CLA. Scale bar for all figures = 75  $\mu\text{m}$ .

The observed morphometric characteristics of *Fusarium* spp. in this study are summarized and compared with previous reports (Table 10).

Table 10-Comparison of morphological characters of the present *Fusarium* isolate with *Fusarium solani* previously described

Morphological characterization	<i>Fusarium</i> isolates under study	<i>Fusarium solani</i> *
<b>Macroscopic characteristic</b>		
Colony colour	White-creamy	White-creamy to white-greyish
Pigmentation	yellowing creamy	Colourless, white-creamy with dark brown zonation
Growth rate (cm) on PDA	5.4±0.8	3.5± 0.2
<b>Microscopic characteristic</b>		
Macroconidia length (µm)	41.4± 2.4	42.0 ±3.0
Macroconidia width (µm)	4.5±0.8	4.7±0.5
Chlamydo spores	Abundant on CLA	Abundant on CLA
Macroconidia septation	3-6	3-7

**Molecular identification of *Fusarium* spp.**

To further support the identification result, the ITS rDNA and ef1 elongation factor of the identified isolates was further amplified by PCR.

PCR amplification of the ITS region with primers ITS1 and ITS4 yielded in an ~570-bp band (Figure 25). The fragment was obtained from all 18 *Fusarium* isolates whereas no product was amplified from negative control. The ITS-region product sequenced from all isolates and compared against NCBI *Fusarium* ID databases revealed that all sequences shared 99% identity with *Fusarium solani* (Table 10).

PCR amplification of the TEF region with primers ef1 and ef2 yielded in a band of 700-bp (Figure 26). The fragment was obtained from all 18 *Fusarium* isolates whereas no product was amplified from negative control. The PCR products were sequenced from all isolates and compared against NCBI *Fusarium*.ID databases revealed that all sequences shared 99% identity with *Fusarium solani* (Table 11).

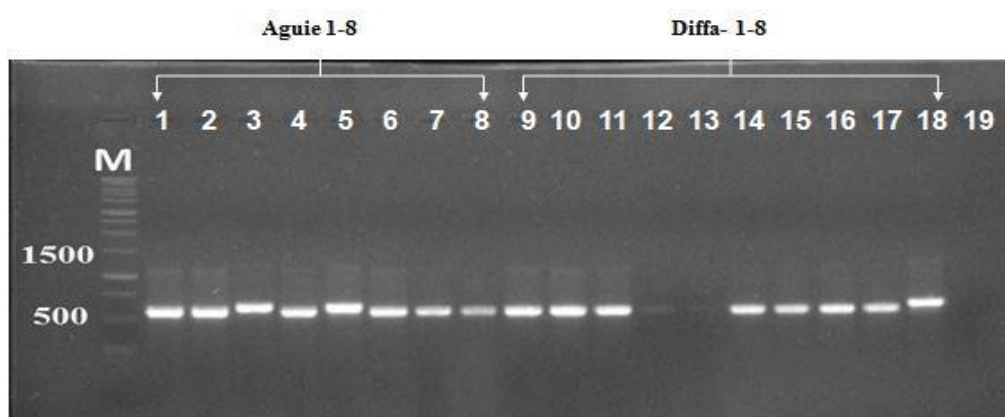


Figure 25: Gel electrophoresis image of the internal transcribed spacer region base pair (bp) products of the *Fusarium* species: M= lanes were loaded with 1kb DNA-Ladder (Promega), lanes 1-8= isolates from Aguie, lanes 9-18 isolates from Diffa, lane 19= negative control with water

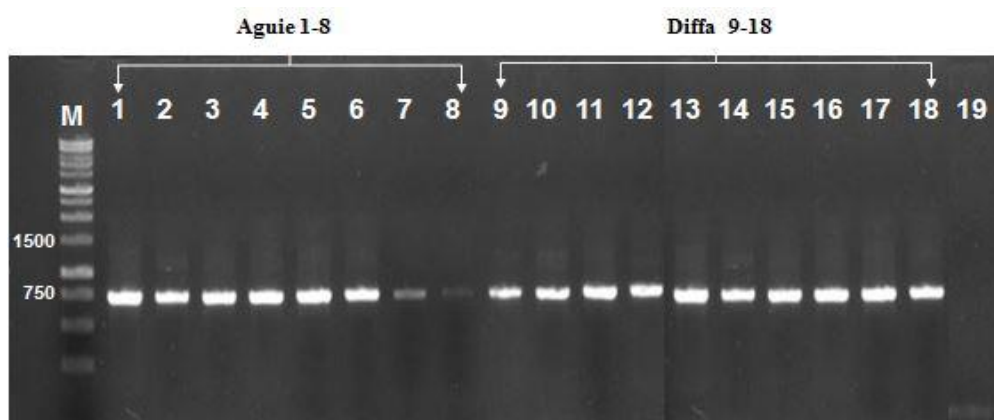


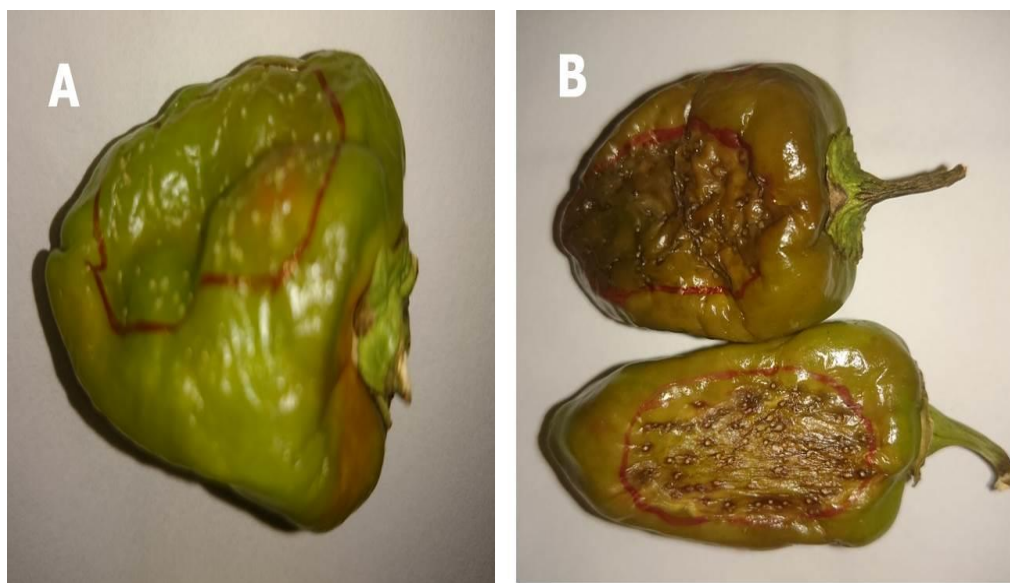
Figure 26: Gel electrophoresis image of TEF- $\alpha$  elongation factor base pair (bp) product of the *Fusarium* species: M= lanes were loaded with 1kb DNA-Ladder (Promega), lanes 1-8= isolates from Aguie, lanes 9-18 isolates from Diffa, lane 19= negative control with control with water.

Table 11-*Similarities in ITS and tef- $\alpha$  of studied isolates and their accession references strains*

District	code	ITS		tef- $\alpha$		
		Isolates	Similarity (%)	Accession number	Similarity (%)	Accession number
Aguie	1		99	KP784419.1	99	HE647946.1
Aguie	2		99	KP784419.1	99	KR816154.1
Aguie	3		99	KP784419.1	99	KM886217.1
Aguie	4		99	KP784419.1	96	HE647946.1
Aguie	5		99	KU528858.1	99	HE647946.1
Aguie	6		99	KU528855.1	99	HE647915.1
Aguie	7		99	KP784419.1	99	HE647942.1
Aguie	8		99	KP784419.1	99	HE647937.1
Diffa	9		99	KF999012.1	99	HE647942.1
Diffa	10		99	KP784419.1	99	HE647924.1
Diffa	11		99	KU296243.1	99	HE647953.1
Diffa	12		99	KP784419.1	99	HE647927.1
Diffa	13		99	KP784419.1	98	KM065871.1
Diffa	14		99	JX868649.1	99	HE647927.1
Diffa	15		99	KP784419.1	99	KR816154.1
Diffa	16		99	KP784419.1	96	HE647946.1
Diffa	17		99	HQ384397.1	97	DQ220248.1
Diffa	18		99	KP784419.1	98	DQ247234.1

### Pathogenicity test on sweet pepper fruit

All the inoculated fruit were infected by the fungus after one week incubation at 25-28°C and 80 % RH in growth chamber. All the isolates successfully colonized the fruits (Figure 27). Initially, the infected parts of the fruits became yellow and soft, and at the end, the whole fruit rotted. No disease symptoms were observed in the control fruits, which remained firm and green. From diseased fruits, *F. solani* was re-isolated on selective medium fulfilling Koch's postulate.



*Figure 27:* Pathogenicity test on green fruits of sweet pepper. (A) Control: no fungal colonization is observed inoculated with sterile water. (B) *F. Solani* development by on fruits leading to fruit rot one week after inoculation.

**Effect of co-infection of *Meloidogyne enterolobii* and *Fusarium solani* on the severity of sudden wilt of sweet pepper plants.**

**Effect of the co-infection of *Meloidogyne* and on *Meloidogyne* abundance**

The results (Figure 28) indicated the overall effect of the co-infection of *M. enterolobii* and *F. solani* on *Meloidogyne* abundance. The highest abundance was recorded in treatment Me whereas there is no *Meloidogyne* in treatment Fs and nMenFs. The analysis of variance (Appendix A) indicated that the effect of treatment on *Meloidogyne* abundance was statistically significant ( $p < 0.000$ ) compared to control.

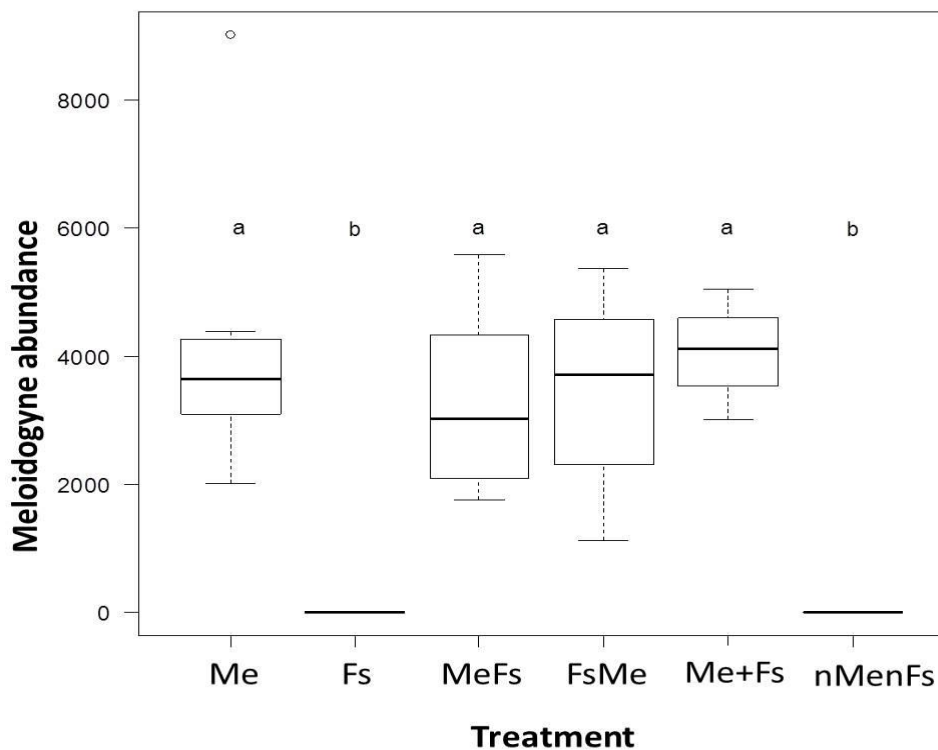


Figure 28: Boxplot of *Meloidogyne* abundance of co-infection of *Meloidogyne* and *Fusarium*. Treatment sharing the same letter was not significantly different (Tukey HSD,  $p < 0.05$ ). Me = *Meloidogyne* alone, Fs = *Fusarium* alone, MeFs = *Meloidogyne* followed *Fusarium* 1week, FsMe = *Fusarium* followed 1week by *Meloidogyne*, Me + Fs = *Meloidogyne* and *Fusarium* simultaneously, nMenFs: no *Meloidogyne*, no *Fusarium* (control)

However, the Tukey HSD post-hoc test at  $p < 0.05$  revealed that there were no significant differences in *Meloidogyne* abundance between the plants inoculated with *Meloidogyne* alone and those with co-infection.

### Effect of the co-infection of *Meloidogyne* and *Fusarium* on *Fusarium* frequency

The results (Figure 29) indicated the overall effect of co-infection of *Meloidogyne* and *Fusarium* on *Fusarium* frequency. The highest frequency was recorded in treatment Fs whereas there is no *Fusarium* in treatment Me and nMenFs (control).

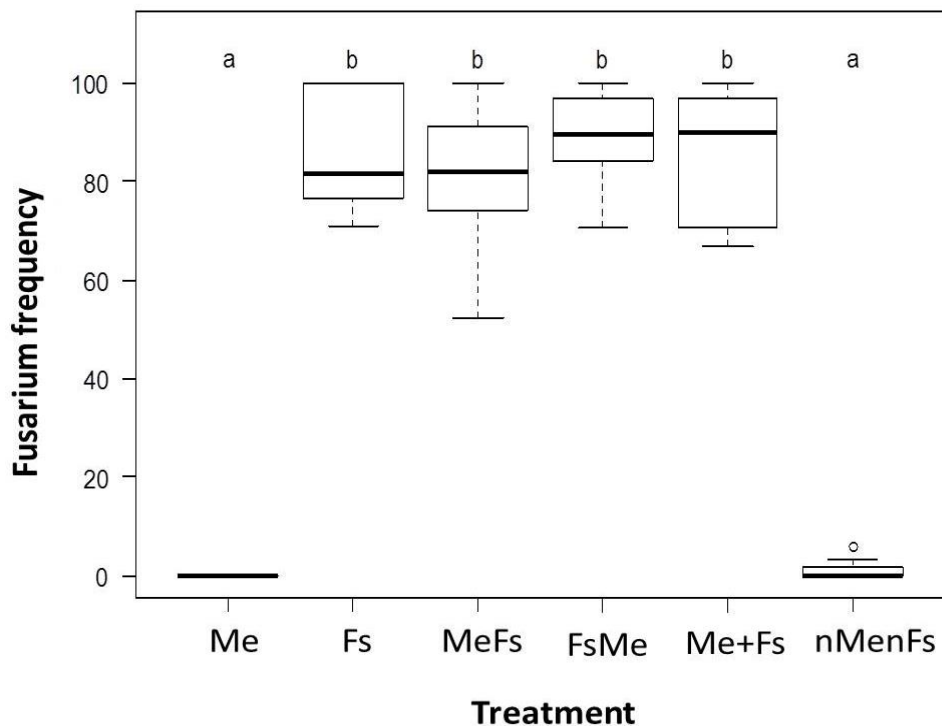


Figure 29: Boxplot of *Fusarium* frequency of co-infection of *Meloidogyne* and *Fusarium*. Treatment sharing the same letter was not significantly different (Tukey HSD,  $p < 0.05$ ). Me = *Meloidogyne* alone, Fs = *Fusarium* alone, MeFs = *Meloidogyne* followed *Fusarium* 1 week, FsMe = *Fusarium* followed 1 week by *Meloidogyne*, Me + Fs = *Meloidogyne* and *Fusarium* simultaneously, nMenFs: no *Meloidogyne*, no *Fusarium* (control)



The analysis of variance (Appendix B) indicated that the effect of treatment on *Fusarium* frequency was statistically significant ( $p < 0.000$ ) compared to control. However, a multiple comparison test (Tukey HSD) test at  $p < 0.05$  revealed that there were no significant differences in *Fusarium* frequency between the plants inoculated with *Fusarium* alone and those with co-infection.

### **Effect of co-infection of *Meloidogyne* and *Fusarium* on the number of yellowing leaves**

The results (Figure 30) indicated that there was a high number of yellowing leaves in the inoculated plants compared to the control. The analysis of variance (Appendix C) indicated that the effect of co-infection of *Meloidogyne* and *Fusarium* on yellowing leaves was statistically significant ( $p < 0.000$ ) compared to control. However, the Tukey-HSD post-hoc pairwise tests indicated test at  $p < 0.05$  indicated that there were no significant differences in number of yellowing leaves on plants inoculated with a single pathogen and those with both pathogens.

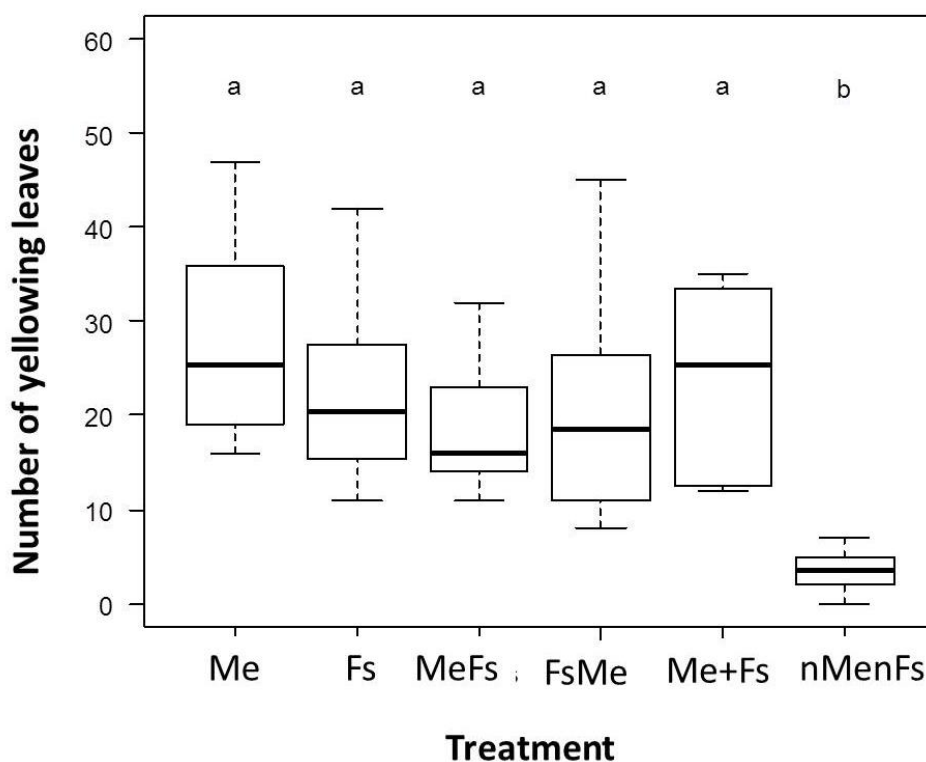


Figure 30: Boxplot of number of yellowing leaves of co-infection of *Meloidogyne* and *Fusarium*. Treatment sharing the same letter was not significantly different (Tukey HSD,  $p < 0.05$ ). Me = *Meloidogyne* alone, Fs = *Fusarium* alone, MeFs = *Meloidogyne* followed *Fusarium* 1week, FsMe = *Fusarium* followed 1week by *Meloidogyne*, Me + Fs = *Meloidogyne* and *Fusarium* simultaneously, nMenFs: no *Meloidogyne*, no *Fusarium* (control).

### Effect of the treatment on total leaves

The results in Figure 31 are a boxplot of the total number of leaves produced by sweet pepper plants after co-infection with *Meloidogyne* and *Fusarium*. The highest total leaves were recorded in the control (nMenFs), 90 leaves compared to the inoculated plants which range from 20 to 58 leaves. The analysis of variance (Appendix D) indicated that the effect of co-infection on total leaves was statistically significant ( $p < 0.000$ ) compared to control. A multiple comparison test (Tukey HSD) test at  $p < 0.05$  revealed that there were

no significant differences in total number of leaves between the plants inoculated with a single pathogen and those with both pathogens.

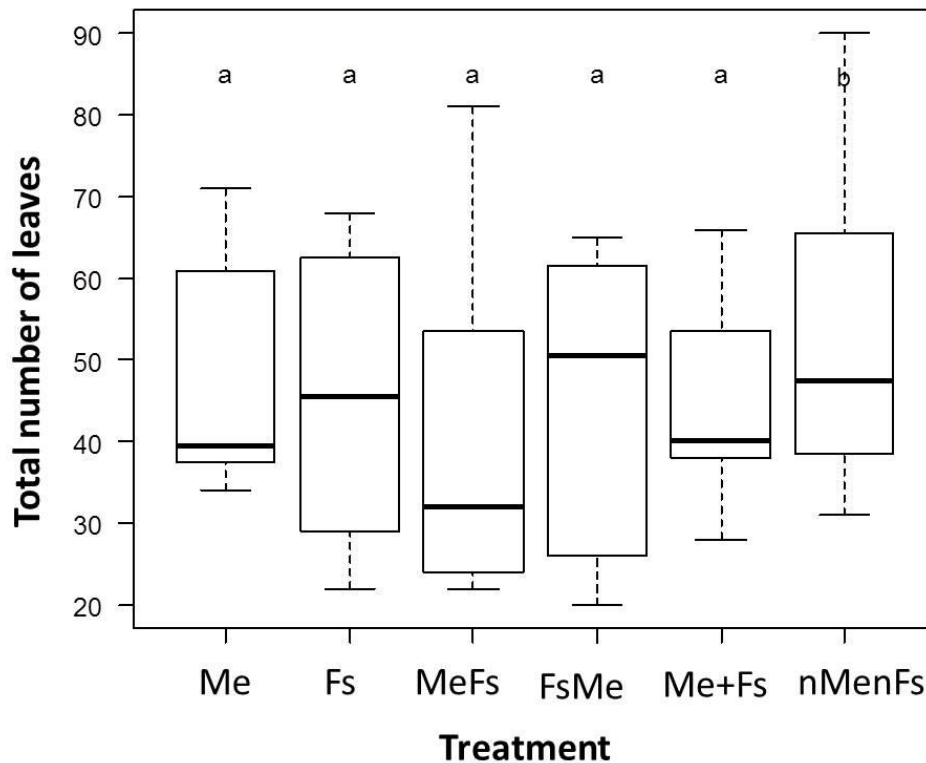


Figure 31: Boxplot of total leaves. Treatment sharing the same letter was not significantly different (Tukey HSD,  $p < 0.05$ ). Me = *Meloidogyne* alone, Fs = *Fusarium* alone, MeFs = *Meloidogyne* followed *Fusarium* 1week, FsMe = *Fusarium* followed 1week by *Meloidogyne*, Me + Fs = *Meloidogyne* and *Fusarium* simultaneously, nMenFs: no *Meloidogyne*, no *Fusarium* (control).

### Effect of temperature and infection of sweet pepper plants by *Meloidogyne* or *Fusarium* on *Meloidogyne* abundance

The results in Figure 32 show the effect of temperature on *Meloidogyne* abundance after co-infection with *M. enterolobii* and *F. solani*. The results (Figure 32) indicated a decrease of *Meloidogyne* abundance with an increase in temperature, with a noticeable reduction between 27-30°C. The

optimum nematode development was observed between 23-25°C. The analysis of variance type III sum squares (Appendix E) shows a significant effect of temperature on *Meloidogyne* abundance. Neither the inoculation with *Fusarium* alone nor the fact that inoculation of *Meloidogyne* or of *Fusarium* was done first or not was significant.

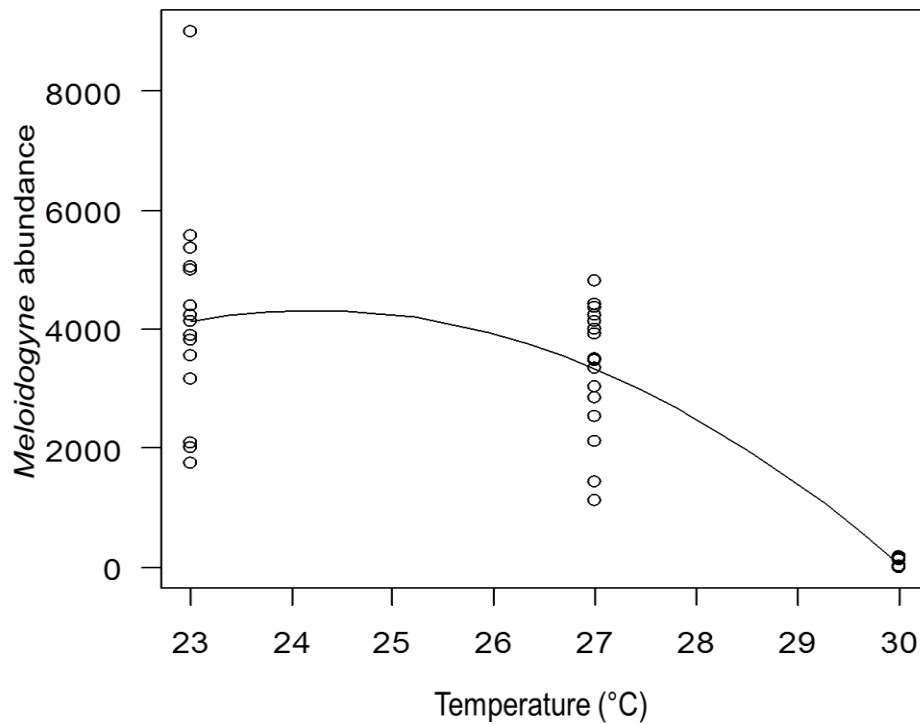


Figure 32: Abundance of *Meloidogyne enterolobii* in soil at different development temperatures

**Effect of temperature and sweet pepper plant infection by *Meloidogyne* or *Fusarium* on *Fusarium* frequency**

The results (Figure 33) indicated that a sharp decrease of *Fusarium* frequency with increased temperature. The analysis of variance type III sums squares (Appendix F) indicated a significant effect of temperature ( $P < 0.000$ )

on the population development of *Fusarium*. The optimum *Fusarium* frequency was observed between 23-26°C.

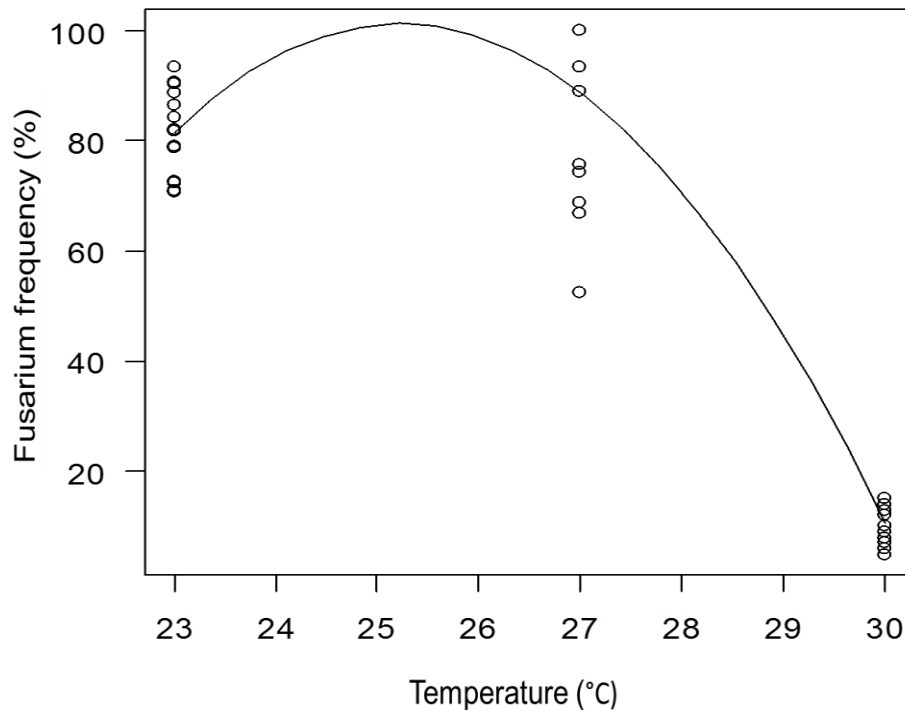


Figure 33: Frequency of *Fusarium solani* in sweet pepper plant tissue at different development temperature

**Effect of co-inoculation of *Meloidogyne* and *Fusarium* on the soil population of the nematode larvae J2 as affected by the temperature**

The results (Figure 34) indicated clearly 2 groups completely separated temperature 30° much lower than the 2 others (23 and 27°C). The analysis of variance type III (Appendix G) shows a statistically significant effect of the interaction between *Meloidogyne* and *Fusarium* (data with both inoculations). The temperature highly affected ( $P < 0.000$ ) the number of *Meloidogyne* larvae J2 in the soil and the *Fusarium* frequency. At 30°C, the number of *Meloidogyne* larvae and the frequency of *Fusarium* were minimal. The highest

population of nematodes in soil and of *Fusarium* in plant tissues was observed between 23 and 27°C.

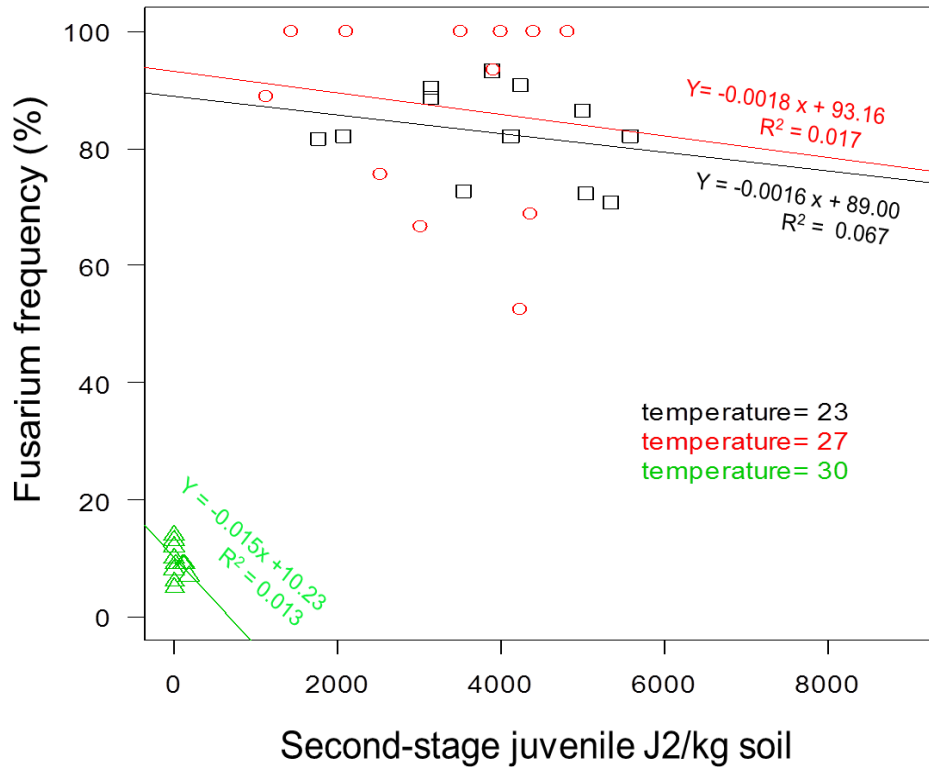


Figure 34: Evolution of the number of J2 larvae of *Meloidogyne enterolobii* and of *Fusarium solani* frequency infection of sweet pepper as affected by temperature

**Perception of farmers on climate variability, adaptations strategies and access to agricultural extension services in Diffa and Aguié**

**Social characteristics of farmers of Diffa and Aguié districts**

The social characteristics of farmers used in this study are presented in Figures 35 to 37. The results (Figure 35) indicate that the respondents were in the age range of 25 to above 70 years. However majority of famers were within the age range of 25 to 50 years in both Diffa and Aguié.

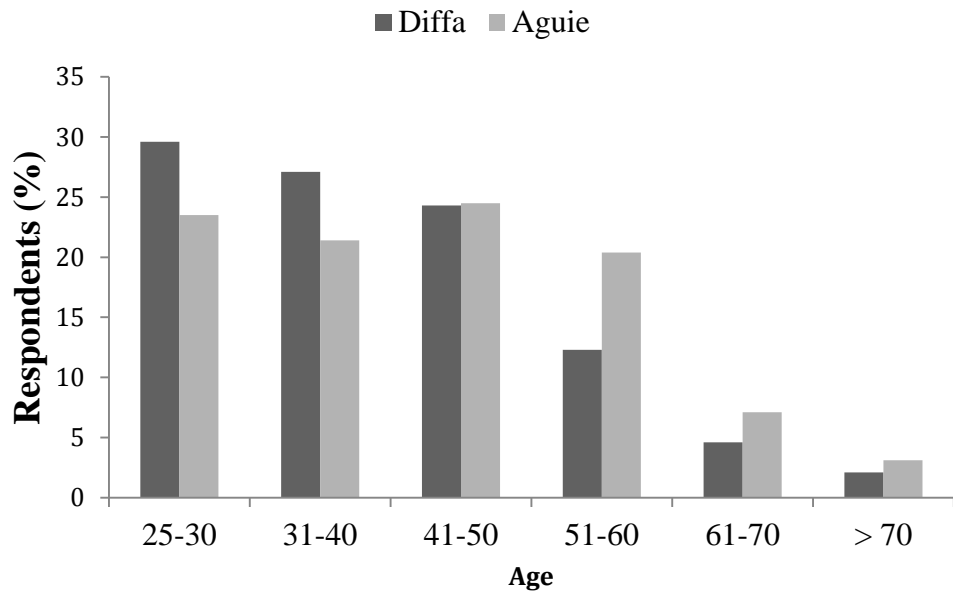


Figure 35: Age of farmers in Diffa and Aguié

Majority of the householders 43% and 41% respectively in Diffa and Aguié had 11-20 years of farming experience (Figure 36).

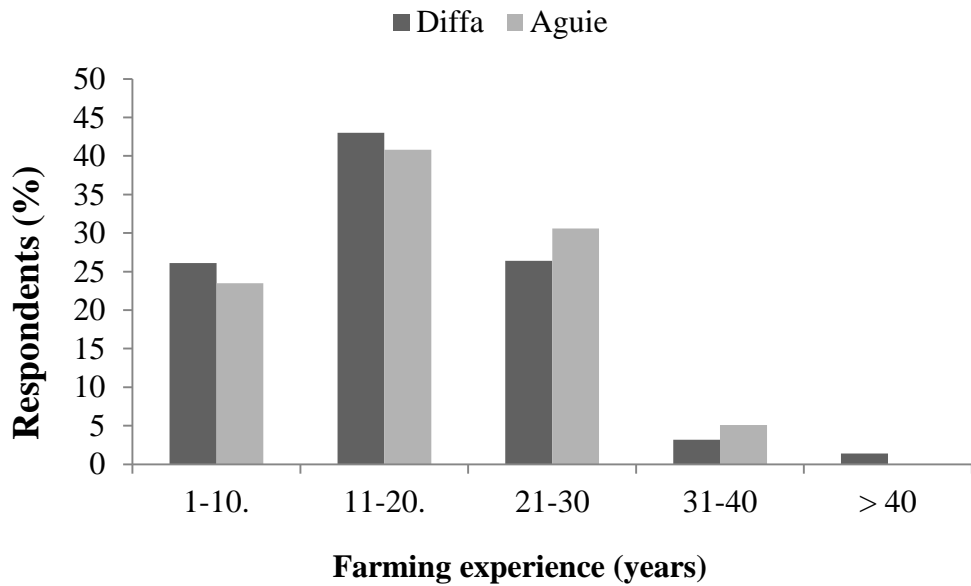


Figure 36: Farming experience of farmers in Diffa and Aguié

In both districts respondents have much in common with respect to education. Most of the farmers (90%) had no formal education in Diffa. However 6% had primary level and 3% secondary level. Similarly in Aguié 72% had no formal education and only 14% and 13% had respectively primary and secondary education level (Figure 37).

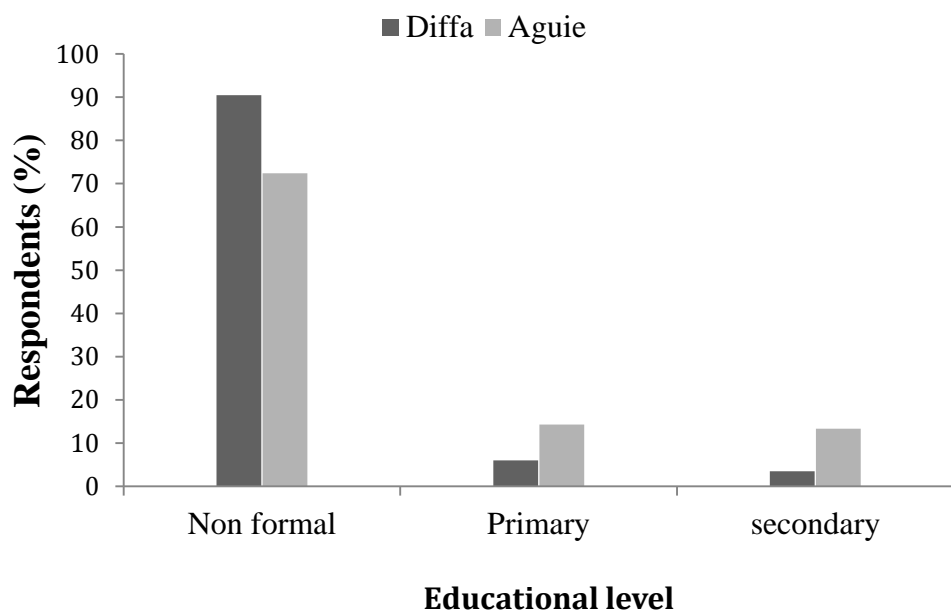


Figure 37: Educational level of farmers in Diffa and Aguié

### Perception of farmers on changes in rainfall patterns

Farmers were asked how the rainfall patterns had changed over the past 20 years. All respondents had observed a number of changes in the rainfall pattern (Figure 38). Most of the farmers claimed that the rainy season started late and stopped early. Majority of respondents 86% in Diffa and 57% in Aguié noted lateness in the onset of rains. On the other hand 66% in Diffa and 55% in Aguié believe that the cessation was earlier. Some differences



emerged in respect of the length of the rainy season and rainfall amount. Majority of farmers (82%) noted a decrease in rainy season length over time in Diffa, in contrast 52% believed an increase in Aguié. Over 77% of farmers also believed that rainfall amount had decreased in Diffa, on the contrary 56% perceived its increase in Aguié. Further analysis on sub-group basis (Figure 39) indicates almost similar results across both districts with some little difference in rainy season length in Diffa. Most of the respondents with more than 20 years of farming experience believed that there has been an increase in the length of the rainy season in Diffa district (Figure 39).

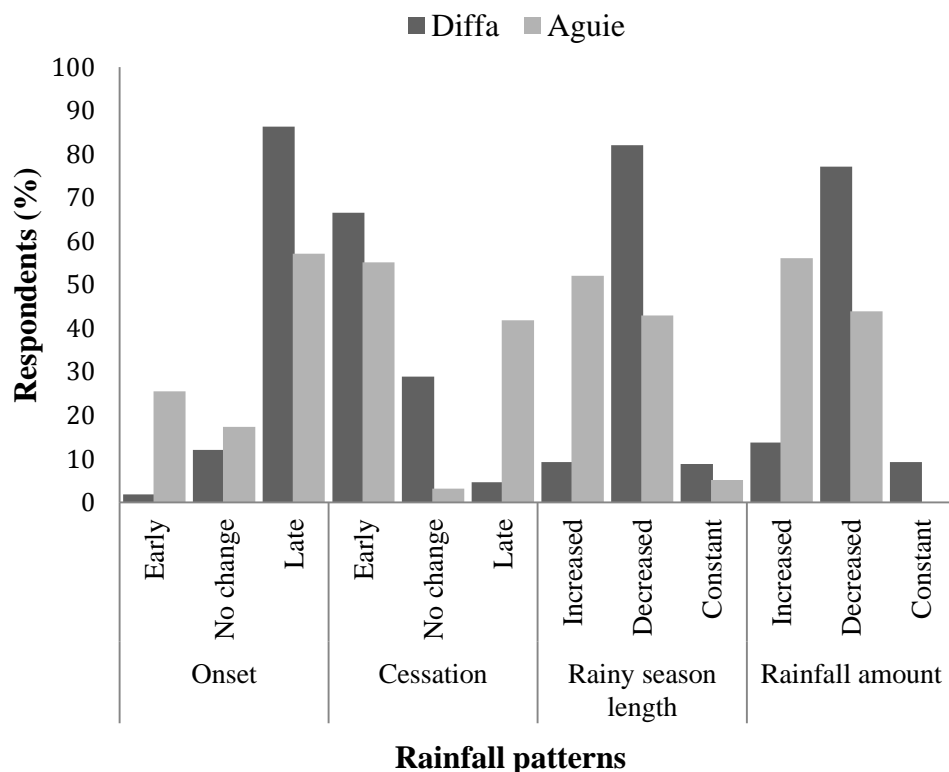


Figure 38: Perception of farmers on changes in rainfall patterns in Diffa and Aguié

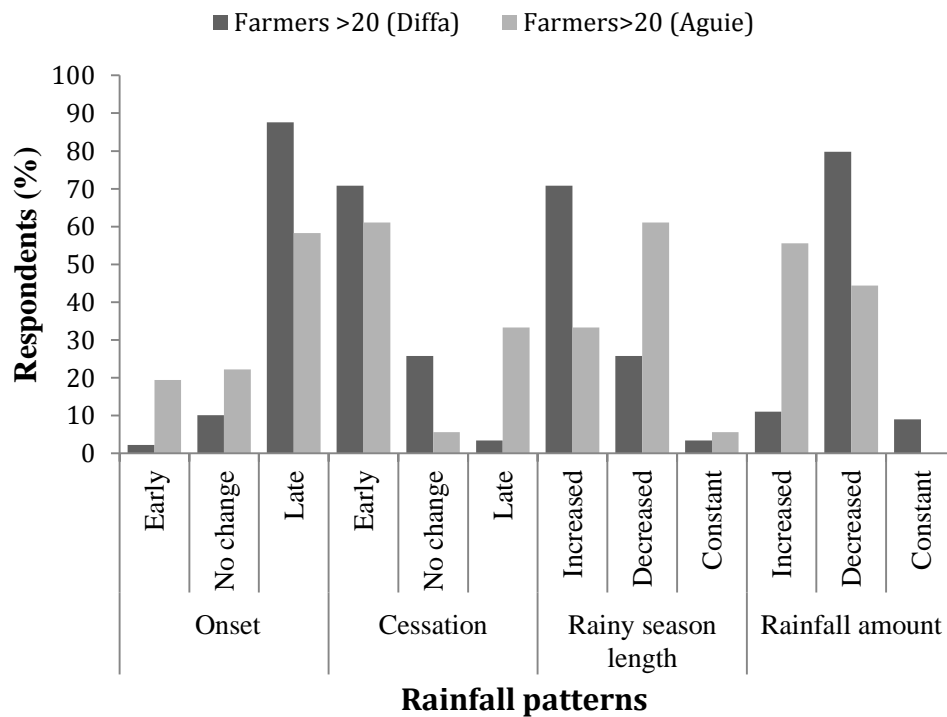


Figure 39: Perception of farmers (with more than 20 years farming experience) on changes in rainfall patterns in Diffa and Aguié

### Perception of farmers on changes in temperature and drought

A large number of smallholder farmers' perceived changes in temperature and drought (Figure 40). Almost 84% noticed an increase of temperature over the last 20 years in Diffa compared to 81% in Aguié. Over 46% of respondents observed an increase in duration of droughts in Diffa compared to 58% in Aguié.

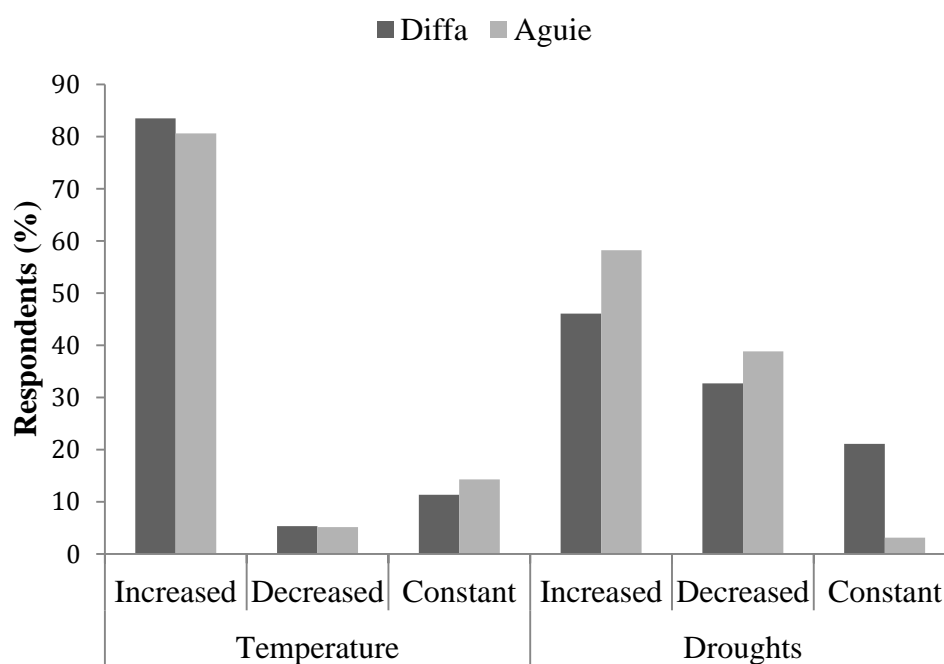
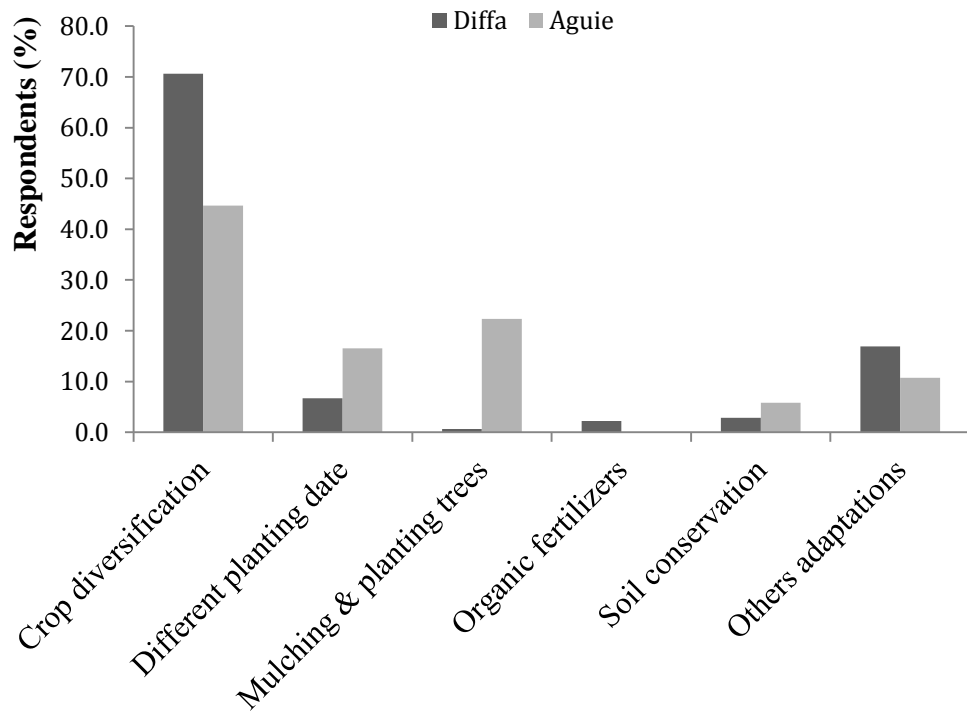


Figure 40: Perception of farmers on changes in temperature and duration of drought in Diffa and Aguié

#### **Farmers' adaptation strategies to climate variability and climate change**

The results (Figure 41) indicate that both farmers in Diffa and Aguié had adopted various adaptation strategies to climate variability. These included crop diversification (76%), different planting dates (3%), planting of trees (0.4%), organic fertilizers (2%) and other strategies like the use of drought resistant crop varieties, increasing frequency of irrigation and changing fields (0.4%) in Diffa compared to 54%, 14%, 11%, 0%, 7% and 13% in Aguié respectively.



#### **Adaptation strategies to climate variability**

*Figure 41:* Adaptation strategies to climate variability for all respondents in Diffa and Aguié

The results Figures 42 and 43 in Diffa and Aguié respectively, indicated that crop diversification was the most predominant adaptation strategy, but increased use of other solutions such as different planting dates, planting trees, use organic fertilizers was observed for young farmers compared to old farmers (Figures 42 and 43). Farmers with less than 20 years of experience are also more likely to adopt more adaptation strategies.

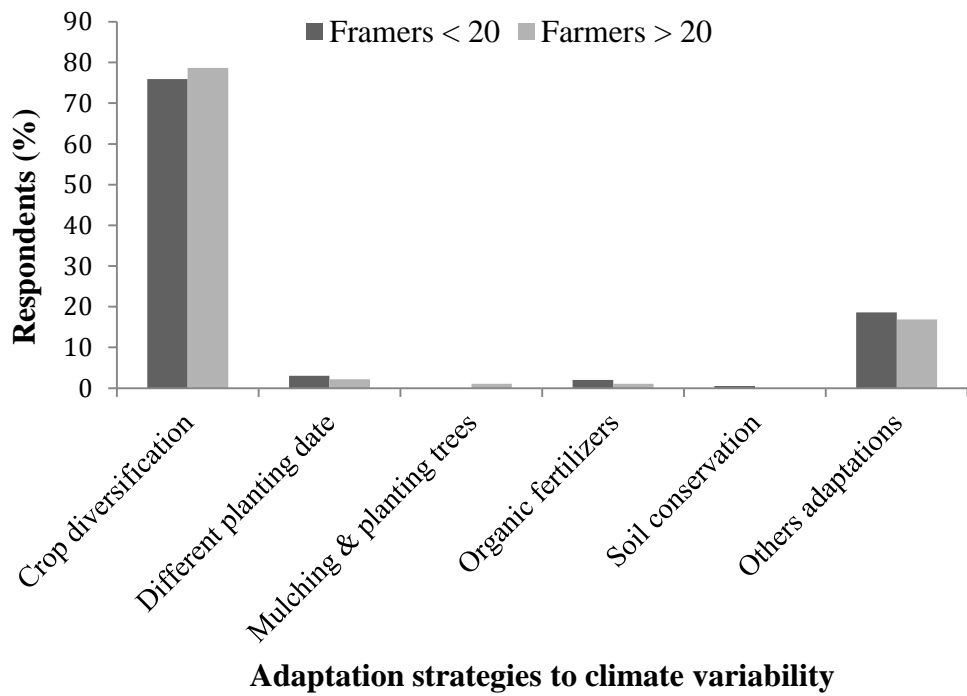


Figure 42: Adaptation strategies to climate variability in Diffa (farmer > 20 years faming experience vs farmers < 20 years)

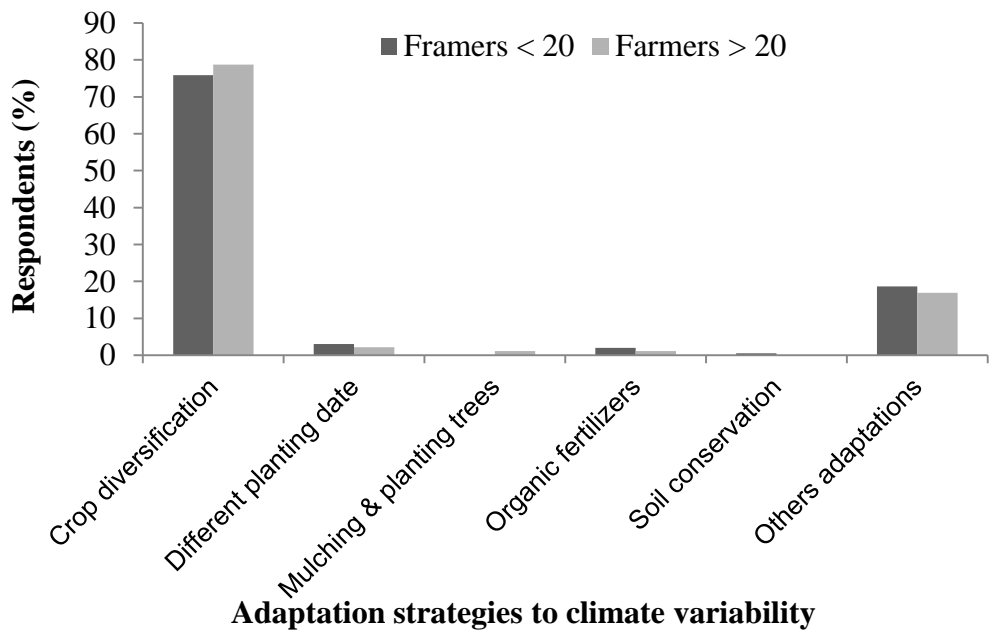
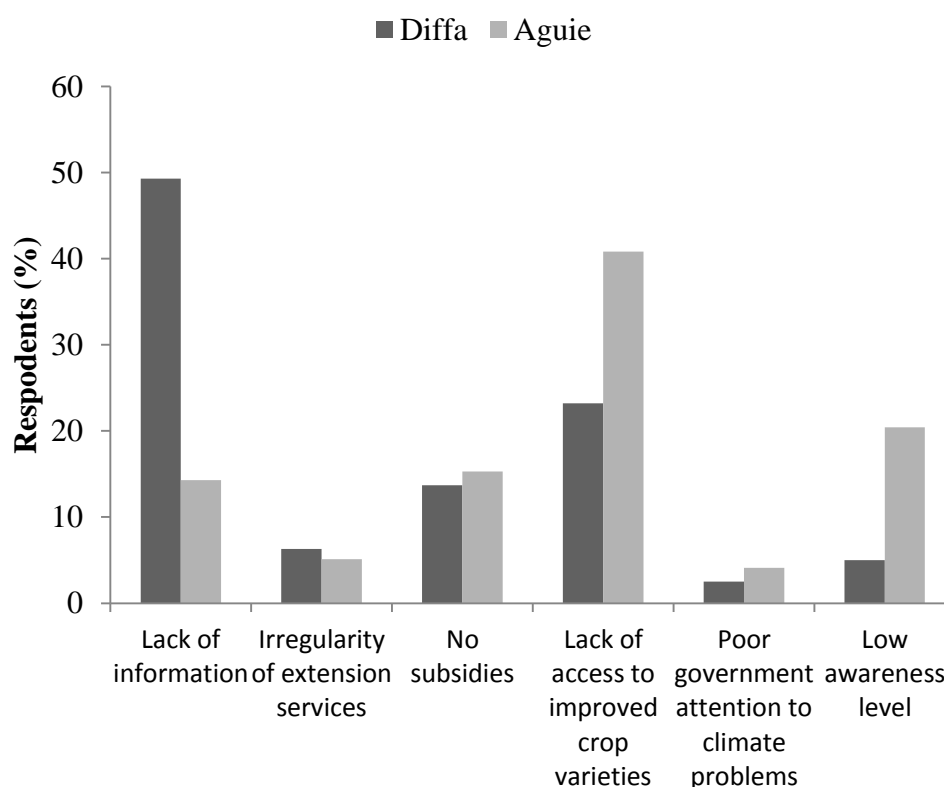


Figure 43: Adaptation strategies to climate change in Aguié (farmer > 20 years faming experience vs farmers < 20 years)

## Barriers to Climate Change Adaptations

The lack of information, irregularity of extension services, no subsidies, lack of access to improved crop varieties, poor government attention to climate problems and low awareness level were the various barriers that prohibit farmers from adopting adaptation strategies effectively (Figure 44).



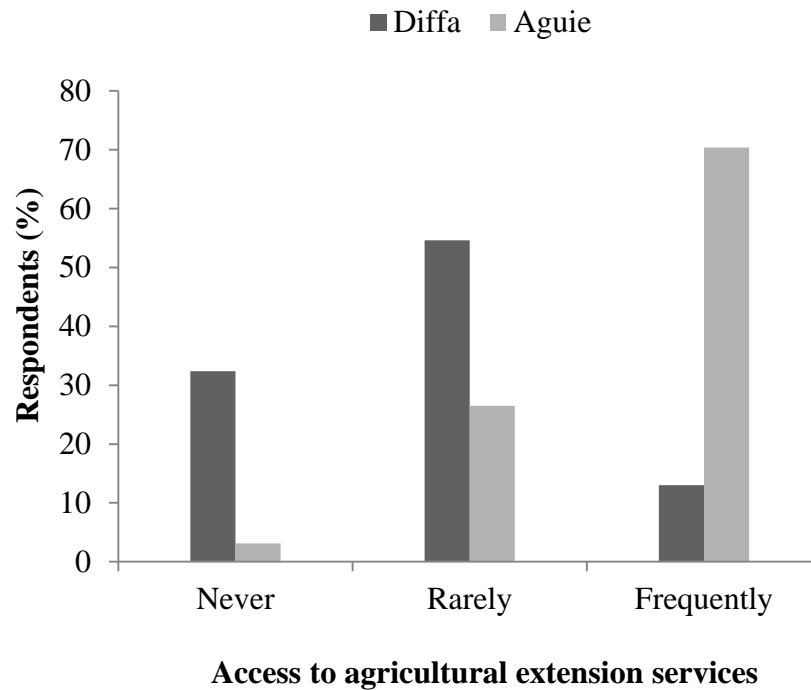
### Barriers to adaptation measures to climate change

*Figure 44:* Barriers to adaptation measures to climate change in Diffa and Aguié

#### Access of farmers to agricultural extension services

The analysis of the frequency of visitation of extension agents by farmers indicated that 32% of the farmers had never been visited by extension agents and 55% had rarely visited in Diffa. In contrast 70% had frequently

contact with extension services and 3% only had no visitation by extension agents in Aguié (Figure 45).



*Figure 45: Access of farmers to agricultural extension services in Diffa and Aguié*

### **Meteorological data analysis**

A simple linear regression was used to depict the linear trends of climatic parameters data over the past 20 years in Diffa and Aguié and is presented in Figures 46 to 53. Statistical significance of the regression equation conducted via t-test ( $\alpha = 0.05$ ) are presented in Table 12 and 13 for Diffa and Aguié, respectively. All trends for onset, cessation, temperature, rainfall amount and rainy season length were either negative or positive for the period of study (1995-2014) in both Diffa and Aguié.

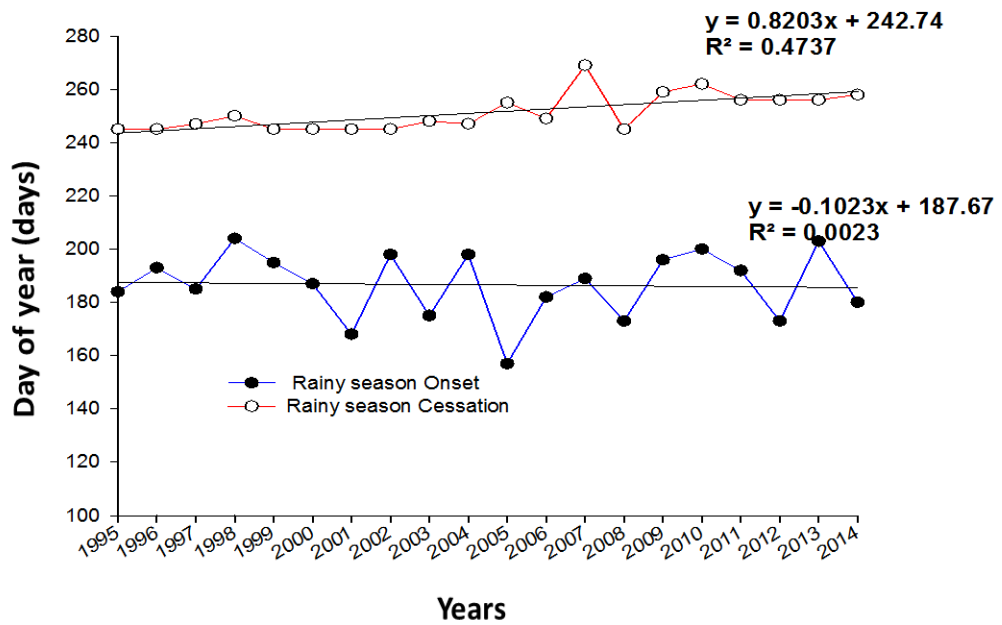


Figure 46: Trend of onset and cessation of the rainy season in Diffa

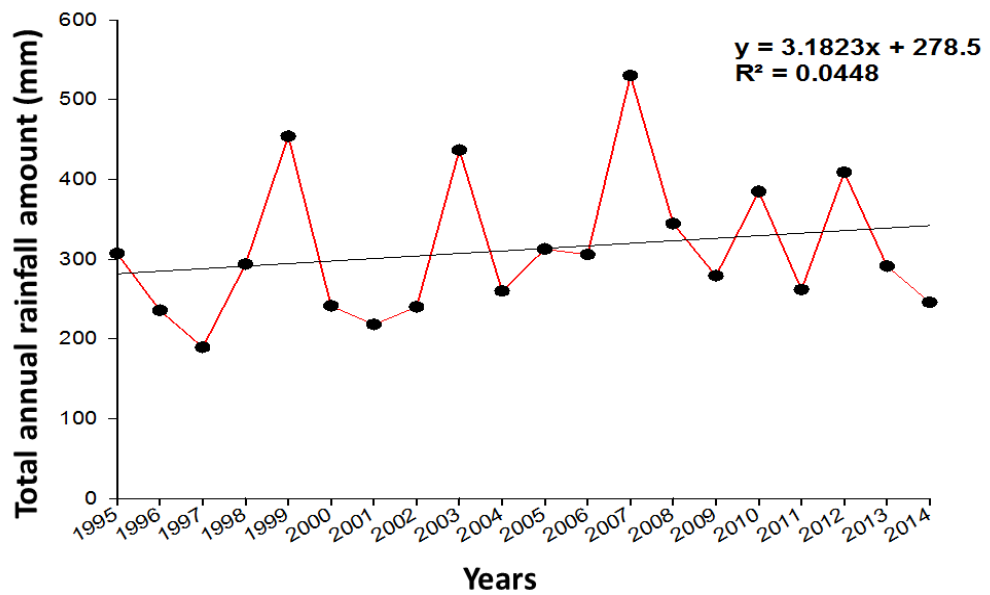


Figure 47: Trend of annual amount of rainfall in Diffa



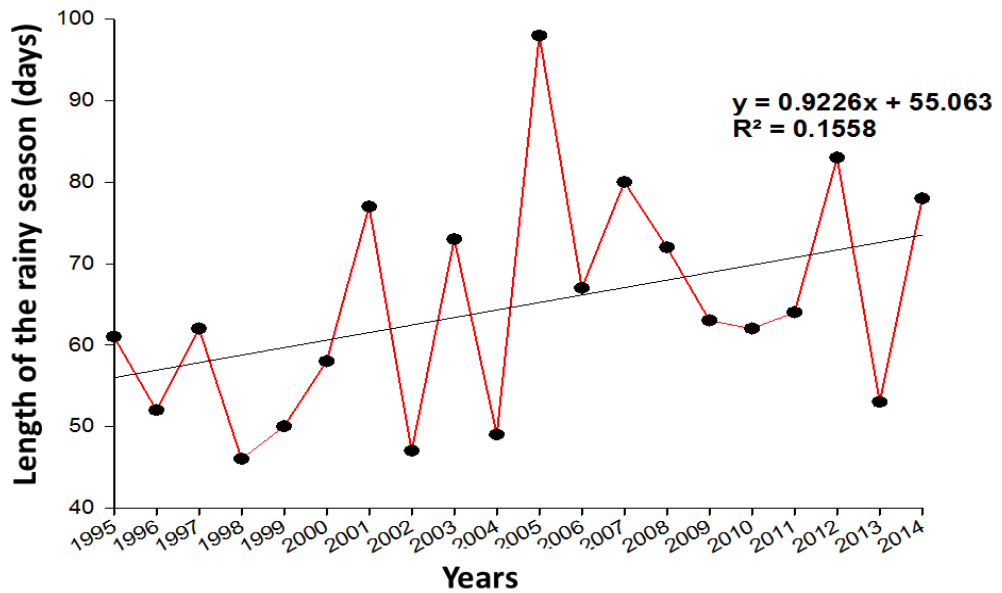


Figure 48: Trend of length of the rainy season in Diffa

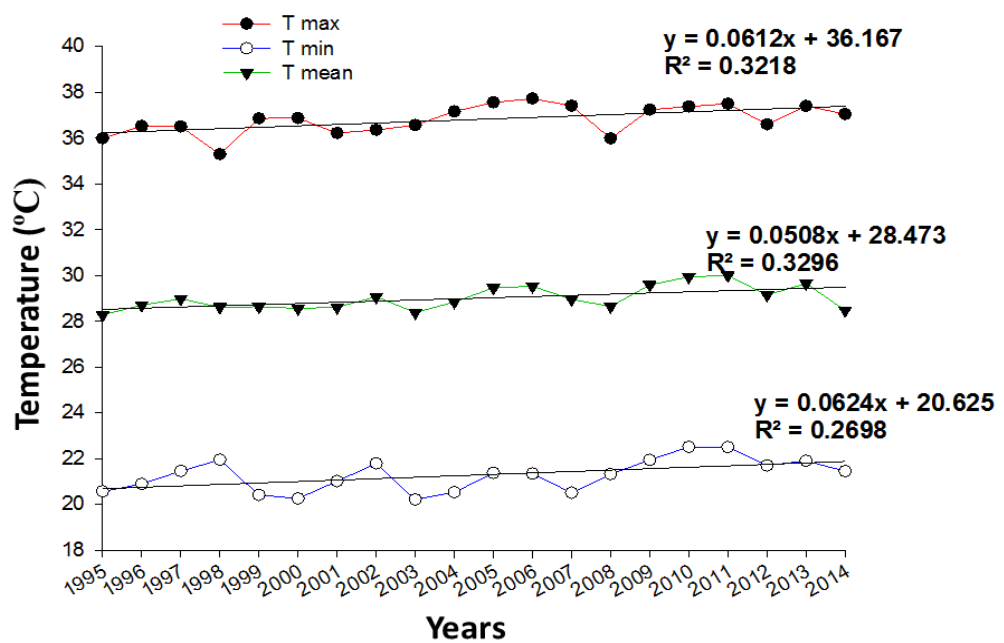


Figure 49: Trend of annual temperature average (minimum, maximum and mean) in Diffa

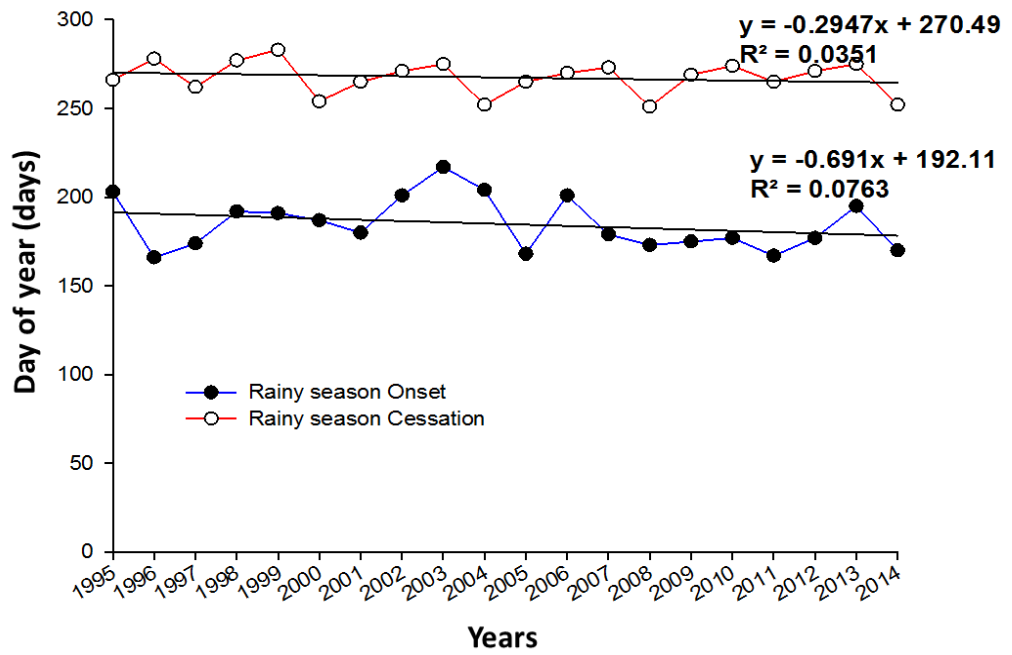


Figure 50: Trend of onset and cessation of the rainy season in Aguié

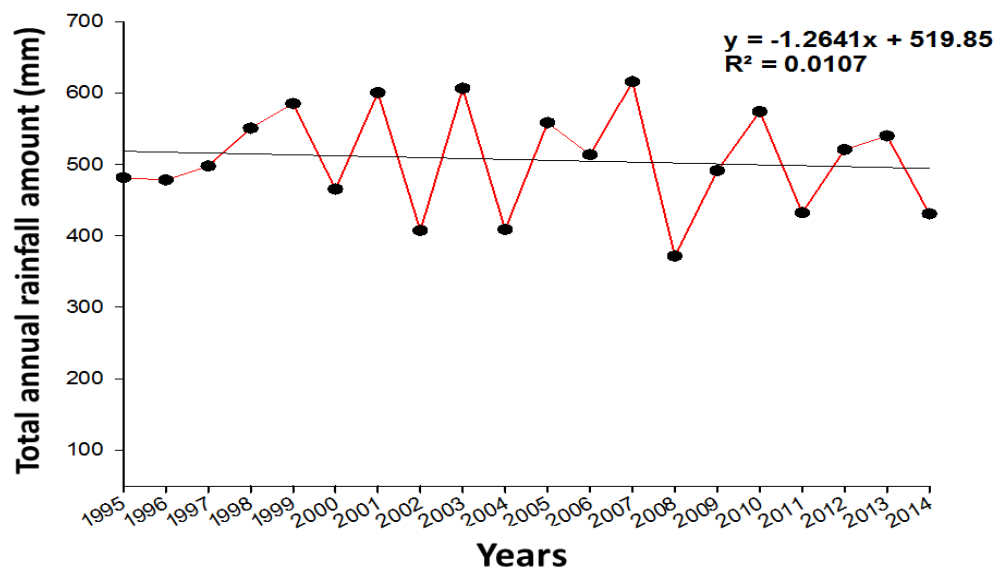


Figure 51: Trend of annual amount of rainfall in Aguié

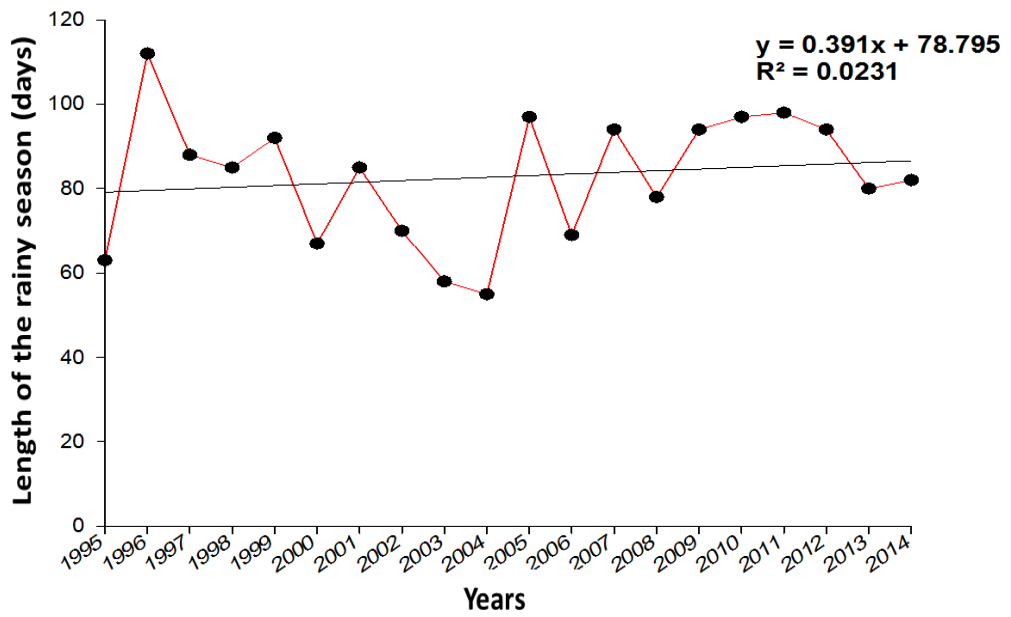


Figure 52: Trend of length of the rainy season in Aguié

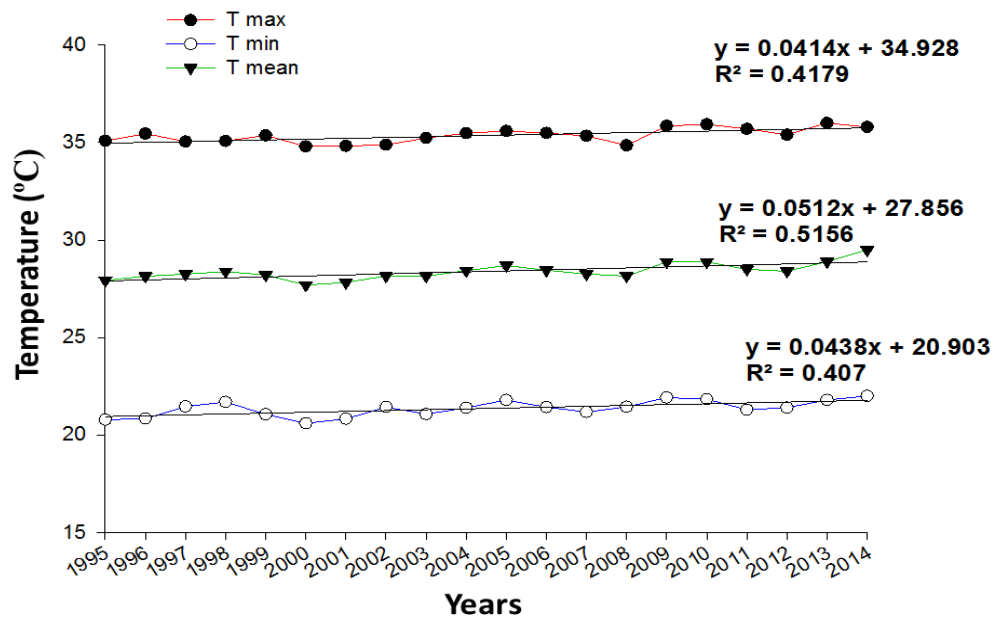


Figure 53: Trend of annual temperature average (minimum, maximum and mean) in Aguié

Table 12: T-test analysis of regression slopes of climatic parameters in Diffa

Parameters	Unit	Mean (1995-2014)	Regression equation	Significance ( <i>p</i> -value)
T max	[°C]	36.8	$Y = 0.06x + 36.15$	0.009
Tmin	[°C]	21.3	$Y = 0.05x + 28.47$	0.019
T mean	[°C]	29	$Y = 0.06x + 20.62$	0.008
Onset	DOY	187	$Y = 0.82x + 242.74$	0.841
Cessation	DOY	251	$Y = -0.10x + 187.67$	0.001
Rainfall amount	mm	311.9	$Y = 3.18x + 278.50$	0.37
Rainy season length	days	65	$Y = 0.92x + 55.06$	0.08

Table 13-T-test analysis of regression slopes of climatic parameters in Aguié

Parameters	Unit	Mean (1995-2014)	Regression equation	Significance ( <i>p</i> -value)
T max	[°C]	35.4	$Y = 0.04x + 34.92$	0.009
Tmin	[°C]	21.4	$Y = 0.04x + 20.90$	0.019
T mean	[°C]	28.4	$Y = 0.05x + 27.85$	0.008
Onset	DOY	185	$Y = -0.69x + 192.11$	0.23
Cessation	DOY	267	$Y = -0.29x + 270.49$	0.42
Rainfall amount	mm	506.6	$Y = -1.26x + 519.85$	0.66
Rainy season length	days	83	$Y = 0.39x + 78.79$	0.53

In Diffa, Figure 46 a positive trend of the onset of rainy season, but the t-test of linear regression (Table 12) shows that there is no significant over the past 20 years. The trend of the cessation of rainy season was negative (Figure 46) and this trend was statistically significant (Table 12). The trends of rainfall amount and the length of the rainy season were positive Figure 47 and 48 respectively, but those trends were statistically significant over the past 20 years in Diffa (Table 12). The trends of temperature (maximum, minimum and mean) in Figure 49 were all positive showing that there is increase temperature over the past 20 years in Diffa. The t-test of regression of temperature indicated that all those trends were statistically significant (Table 12).

In Aguié, the trend of the onset and cessation of the rainy season were all negative (Figure 50) indicating that there was the onset was late and the cessation was early, but the t-test (Table 13) shows that is no significance in onset and cessation over the past 20 years in Aguié. The trends of the length of rainy season (Figure 51) and rainfall amount (figure 52) were positive and negative respectively but not statistically significant. The trends of temperature in Aguié (Figure 53) showed a slight increase and the result was statistically significant during the period of 1995-2014 (Table 13).

## CHAPTER FIVE

### DISCUSSIONS

#### **Distribution, occurrence and importance of *Meloidogyne* spp. sweet pepper in Diffa and Aguié**

During the survey in Diffa and Aguié, it was observed that the growth of sweet pepper was highly variable; plants were stunted and severely galled. The dense presence of giant galls and rotted roots may have drastically reduced water uptake, mainly during the flowering period leading to observed symptoms. A similar observation in guava decline due to *M. enterolobii* infection has been reported (Gomes, Souza, Silveira, & Almeida, 2014). Water stress and growth stage of plants have been reported to aggravate yellowing and wilting symptoms (Gomes et al. 2011).

The results indicated that sweet pepper was heavily infected by *Meloidogyne* spp. at all the sites. The incidence of *Meloidogyne* spp. was 100% at all sites in both, Diffa and Aguié. The densities of *Meloidogyne* species and root-knot gall index were high. *Meloidogyne* reproductions parameters i.e. gall per root system, nematode population were statistically variable among the sampling sites. (Davis et al., 2003) reported that variability of in number of galls on roots was influenced by the nematode reproduction rate) or the final population of nematodes at harvest (Kamran et al., 2011).

Furthermore, variability in *Meloidogyne* spp. populations was observed in both districts. These results are similar to that of by Haougui et al. (2013).

This variability in *Meloidogyne* spp. could be due to the soil texture. It has been reported that variability in *Meloidogyne* spp. population density is influenced by soil texture (Ogbugi, 2004). In their study, Noe and Sikora (1990) indicated that the density of plant parasitic nematodes might be very high in sandy soil. Sweet peppers grown in sandy soils are heavily infested by RKNs (Haougui, 1999). In this study, soils collected from sweet pepper were sandy soil could favour *Meloidogyne* reproduction. The mobility of the nematodes depended upon both the soil type and soil water flow (Fujimoto et al., 2010). Soil type influenced penetration of root by nematodes (Koenning & Barker, 1995), nutrient balance (Melakeberhan, 2002)

Another factor for high density of *Meloidogyne* spp. in sampling sites may be due to the mono-cropping systems. The information collected from growers of sweet pepper indicated that the entire fields observed had been under monoculture for many years. The practice of cropping in monoculture with susceptible crops was very common in these areas and that may have increase the RKN infestation (McSorley, 1999).

A number of plant parasitic nematodes can infect sweet pepper. In his study, Haougui (1999) reported 20 genera of plant parasitic nematodes on vegetables including sweet pepper while detailed survey of sweet pepper listed 12 genera (Djibey, (2012). Similarly, Haougui et al., (2013) listed 7 genera; Zakari (2008) listed 9 genera. In our study ten (10) genera of parasitic nematodes were identified, including *Meloidogyne*, *Tylenchorynchus*, *Helicotylenchus*, *Criconemella*, *Xiphinema*, *Scutellonema*, *Hoplolaimus*, *Rotylenchulus*, *Pratylenchus* and *Trichodorus*. On the basis of frequency and

abundance, *Meloidogyne* spp. was most predominant and the major plant-parasitic nematodes at all the sweet pepper growing sites in both Diffa and Aguié.

Around the world, the genus *Meloidogyne* has been reported as the most important nematode causing severe damage in sweet pepper. Khan (2008) reported that *M. incognita* and *M. javanica* were the most damaging and widespread of all the nematode pests of chili and sweet pepper in the savanna zone of northern Nigeria. Dalhatu et al. (2014) and Bachie (2015) found a high population of *M. incognita* infesting sweet pepper (*Capsicum annuum*) in southern California. Kiewnick et al. (2009) reported that *M. enterolobii* infects pepper cultivars considered resistant to *M. incognita*, *M. javanica* and *M. arenaria*.

#### **Occurrence *Fusarium* spp. associated with sweet pepper in Diffa and Aguié**

The *Fusarium* occurrence was highly prevalent in both districts. Disease symptoms including wilting without yellowing were observed on diseased plants in comparison with healthy plants. In fact on the fields, near 30% of plants examined were wilted. *Fusarium* wilt is considered as the major devastating and destructive disease affecting crop production of pepper (Black, 1991). In Almeria, Spain, wilting symptoms were observed without yellowing of leaves and stunting of sweet pepper plants grown in nursery with *F. oxysporum* f. sp. *capsici* identified as the causal agent (Lomas-Cano et al., 2014). From previous survey in Niger, wilting of vegetable crops including sweet pepper caused by nematodes and *Fusarium* was also reported by



Haougui et al. (2013), but there *F. oxysporum* was identified as the causal agent of the wilting disease.

In this present survey, beside wilting and drying plant symptoms, in some fields, fruit rot was also observed. Fungal isolates from the rotten stem and roots of sweet pepper were identified as *F. solani*. Stem and fruit rots as well as wilting and death symptoms caused by *F. solani* were also reported by Lamb et al. (2001) in sweet pepper grown in greenhouses in south Florida (U.S). Ramdial and Rampersand (2010) have reported severe fruit rot symptom of sweet pepper infected by *F. solani* in Trinidad resulting in a disease incidence of 80% with a yield loss of 40 to 60%.

#### **Identification of *Meloidogyne* spp. and *Fusarium* spp. associated with sweet pepper**

Characterization of the seven isolates of *Meloidogyne* spp. was carried out based on the morphological observations of the perineal pattern and morphometric measurements of second stage juveniles and males. The first six (1-6) isolates were identified as *M. enterolobii*. The perineal pattern and the morphometrics of second-stages juveniles from Niger isolates matched with those reported in the original description of *M. enterolobii* (Yang & Eisenback, 1983). However, some of the morphological characters of diagnostic significance such as body length, showed lower range values in isolate 1 and 6 (Table 6), but the ranges of these characters overlapped. Furthermore, the result agrees with those of Karsen, Liao, Kan, Van Heese

and Den Nijs (2012), where the mean body length of second-stage was  $408 \pm 18 \mu\text{m}$  and the range was (380–442  $\mu\text{m}$ ). The for this study was 395 to 410  $\mu\text{m}$

Regarding the male measurements, the results matched with those reported in the original description of *M. enterolobii* (Yang & Eisenback, 1983; Karsen et al. (2012), where the average body length of males was  $1230 \pm 316\mu\text{m}$  and the range was (865–1667 $\mu\text{m}$ ). Even the morphological characteristics of second-stage juveniles and males of this study matched the original description of *M. enterolobii* (Yang & Eisenback 1983; Karsen et al., 2012), with some differences observed for certain characters among the isolates. Nevertheless the ranges of these characters overlapped; hence the differences between mean values can be attributed to the variability within and among isolates.

The perineal pattern of isolate 7 and the morphometrics of second stage juveniles were identified as *M. incognita* and were similar to those reported by Brito et al., (2004). It was noted that for morphological characteristics, it was difficult to distinguish between *M. enterolobii* and *M. incognita*. Any identification based on morphological characters in mixed populations could be inaccurate and not reliable. Morphological characterization should therefore be supported by molecular characterization.

Accurate identification of *Meloidogyne* spp. is of significant importance in terms of developing an effective cropping system and management strategies. The PCR reaction amplification using the primers set C2F3 and 1108 was used to amplify the intergenic region between cytochrome oxidase subunit II gene (CO II) and large subunit of rRNA gene (rRNA) of the

mitochondrial genome (Powers & Harris, 1983). Different bands sizes were detected in the mitochondrial genome of *Meloidogyne* spp. The 705~bp amplicon was confirmed as specific for *M. enterolobii*. The results were similar to those reported by Brito et al. (2004) (705 bp in a sample from Florida), Zhuo et al. (2010) (705 bp in a sample from China), and Iwahori et al. (2009) (705 bp in a sample from Vietnam).

The identification of *Meloidogyne enterolobii* on sweet pepper is the first report of its report in Niger. It is important to note that *M. enterolobii* is considered as the most aggressive known tropical root-knot nematode species (Brito et al., 2004). Furthermore, various studies (Carneiro et al., 2006; Kiewnick et al. 2008; Kiewnick et al., 2009) have reported the presence of this pathogen in vegetable crops considered resistant to *M. incognita*, *M. javanica* and *M. arenaria* (Neal, 1889) Chitwood, 1949). Due to its aggressiveness (Hussey & Janssen 2002), it has the ability to overcome the resistance of tomato and pepper genotypes. Furthermore, *M. enterolobii* can also infect trees (Gomez et al., 2014). This created awareness for farmers on the presence of this pathogen which influence their decision on crops to grow and management practices to adopt when growing sweet pepper.

The 1.6kb produced in isolate 7 was sequenced and comparing to NCBI databases revealed that all sequences shared 100% identity with *M. incognita*. The *Hinf I* digestion of 1.6k generated two fragments of 1.2 and 0.37 kb. Similar work has been conducted on identification of *Meloidogyne* species (Powers et al., 2005) using C2F3/1108 primers. In their study, they

found the PCR amplification of was 1.5kb and the *Hinf I* digestion generated two bands, identified as *M. incognita* was similar to their report.

Morphological characterization of *Fusarium* spp. was performed mainly based on distinctive characters of the shapes and sizes of macro- and microconidia, presence and absence of chlamydospores as well as colony appearances, pigmentations and growth rates on agar media as described by Leslie and Summerell (2006). In this study, *F. solani* produced white aerial mycelia, cream to yellowish colonies on PDA. The microconidia were short, zig-zag chains or false heads, obovoid or clavate with a flattened base, and macroconidia were sparse, straight to slightly curve. These morphological characteristics of *F. solani* isolates have earlier also been reported by Hafizi et al. (2013).

Analyses of rDNA sequences constitute an important complement of the morphological criteria needed for precise fungal identification (Guarro et al., 1999). The results of the 18S rDNA analysis showed that all 18 *Fusarium* sp. isolates shared 99% identity with *F. solani*. In a similar study, Zarrin et al. (2016) have also successfully identified *F. solani* based on internal transcribed spacer (ITS) region amplification. Another tool for a reliable identification of *Fusarium* spp. is based on the sequence information of the translation elongation factor 1 $\alpha$  (TEF-1 $\alpha$ ) gene (Nitschke et al., 2009; Geiser et al., 2004). In this study, the comparisons of TEF-1 $\alpha$  gene of all the 18 isolates sequences with the cognate sequences available in NCBI databases revealed 99% sequence identity to *F. solani*. Hence both morphological and molecular characteristics supported identification of *F. solani*.

In this study, *Fusarium* recovered from sweet pepper was identified as *F. solani* based on morphological and molecular analyses, while previous studies found *Fusarium oxysporum* (Haougui et al. 2013). *Fusarium solani* was identified as the causal agent of stem and fruit rot of sweet pepper. The use of resistant cultivars is the most effective wilt disease management including *Fusarium* wilts. However, efforts must be done to control the disease with respect to the occurrence of the two species due to the lack of resistant cultivars in Diffa region. Furthermore, since *F. solani* is known to be seedborne pathogen (Vikas and Varma, 2015; Mehl and Epstein (2007), farmers in Diffa and Aguié who do grow their own seeds collected from the field, should stop doing so in order to reduce the dissemination of disease.

**Effect of temperature and co-infection of *Meloidogyne enterolobii* and *Fusarium solani* on the severity of sudden wilt of sweet pepper plants in Niger**

The results of single and combined inoculation of sweet pepper with *M. enterolobii* and *Fusarium solani* were not significantly different in terms of pathogen reproduction and sweet pepper sudden death symptoms. Single or combined inoculation of the fungus and/ or the nematode resulted in significant increase of yellowing leaves and high nematode and fungal reproduction in inoculated plants in comparison to the uninoculated plants. However *F. solani* or *M. enterolobii*, alone or in mixed infections were not able to induce on sweet pepper the sudden death syndrome observed in the field. This observation could be due to the inoculum density of the

*Meloidogyne* and *Fusarium*. Starr et al. (1989) studied the interaction of *Meloidogyne incognita* and *Fusarium Oxysporum f. sp. vasinfectum* on cotton and found that both pathogens had effect on cotton mortality, but no interaction was observed at lower nematode populations or at higher populations of *Fusarium*. Furthermore, in the field, infected plants have challenging environmental stresses (temperature and soil moisture variations) and consequently, develop wilting symptoms quickly than infected plants growing in constant good soil moisture conditions (Husain & Ghaffar, 1995, Wheeler et al., 1991).

Nevertheless, in some many studies, more detrimental effect of combined inoculation of *Fusarium sp.* and *Meloidogyne* spp. on growth parameters of the host was observed at controlled conditions (Siddiqi & Husain, 1991; Bertrand, Nunaebz, & Saraha, 2000; Gomes et al., 2014). Our experiments using realistic inocula densities (2000 J2/pot for *M. enterolobii* and 20 ml 10<sup>6</sup> spores/pot for *F. solani*) and being limited in time did not support these results. Thus in the eight-week growth chamber experiment, there is no conclusions evidence on the effect of joint infection of *M. enterolobii* and *F. solani* on the development of sudden death symptom of sweet pepper.

The results in this study however provide the evidence that *Meloidogyne* abundance and *Fusarium* frequency were greatly affected by temperature. The logistic model provided excellent fit curves to indicate a decreased of *Meloidogyne* abundance and *Fusarium* frequency with increasing temperature. *Meloidogyne enterolobii* was likely to reproduce abundantly in

moderate temperature ranges of 23 -27°C, and less as the temperature increased to 30°C. These moderate temperatures prevail on the field during the sweet pepper production period, and may favour nematode infection and reproduction. The results are in accordance with previous studies (Guiran & Ritter, 1979; Saremi & Burgess, 2000) who reported that the development of the pathogens is favoured by temperatures and humidity. According to France and Abawi (1994) symptoms of *Fusarium* wilt were detected earlier and the rate of disease development was higher when plants infected by *M. incognita* were incubated at 27°C than those incubated at 19°C. Ploeg and Maris (1999) studied the effect of temperature on the duration of the life cycle of *Meloidogyne incognita* population. They revealed that *M. incognita* completed its life cycle on tomato at the average soil temperature of 16.2 and 30°C.

Many researchers have investigated the combined infection of *Meloidogyne* spp. and *Fusarium* spp. on the development of the nematode and the fungus. The establishment of *Fusarium* spp. through wounds caused by nematodes accelerates the degradation of plant host and thus form disease complexes (Messiaen et al., 1991; Whitehead, 1998; Gurr, McPherson, & Browle, 1992). The disease complex involved in guava decline are related to prior infection from plants parasitized by *M. enterolobii*, which provides temporary source of nutrients, allowing the fungus to grow and increase its capacity to infect the guavas (Gomes et al., 2011). These findings contradicted the results of this study where there was no difference in the development of *M. enterolobii* and *F. solani* on sweet pepper irrespective of the inoculation

sequence (*Meloidogyne* first or second or the both pathogens at the same time).

**Survey for the perception of farmers on meteorological events, adaptations strategies and access to extensions service in Diffa and Aguié.**

The investigation indicated that the majority of the farmers were in the age classes above 25 years in both Diffa and Aguié. Furthermore, most of them had between 11 and 40 years farming experience. Hence, they should be able to give credible information on climatic variability and on its impact in the region. However, it was evident that generally their level of education was low. Schooling level could influence appreciation the ability to appreciate weather patterns and adaptations. Maddison (2007) reported a positive relationship between the education level of the household head and adaptation to climate. Farmers with higher levels of education are more likely to adapt better to climate change. Benor, Harrison and Barter (1997) reported that education is important in creating positive mental attitude towards adoption of modern farming innovations. Promoting improved access to (better-quality) formal education is thus a way to increase adaptive capacity (Wamsler, 2012).

Regardless of agroecological zones, farmers claimed the rainy season started later and stopped earlier, whereas statistically the onset trend was negative, i.e. rainy season tends to start earlier, but the trend is not significant. This was in strong contrast with 86% of farmers who indicated a slight delay in onset. Controversially, the positive and statistically significant cessation trend was in line with the perception of 66% of farmers who claimed that cessation was earlier over the last 20 years. With regards to the length of the



rainy season as well as rainfall amount, over 77% of respondents perceived a decrease in length of growing season and rainfall amount.

The meteorological data indicated that these parameters were constant within the observation period. Similarly the rainy season tends to start earlier in Aguié, but this was not in agreement with the majority of farmers' perceptions (57%) who indicated a later onset. The sub-group analysis of farmers with over 20 years of farming experience, indicated an increase of rainy season length and a decrease of rainfall amount in Diffa, meanwhile in Aguié a decreased length of growing season and an increase of rainfall amount were noted. In both districts, these farmers' perceptions were not supported by the meteorological data.

There was a large discrepancy between the meteorological data measurements of rainfall and the perceived weather patterns in both districts. The perceived variations in rainfall patterns could be explained by the difference in the appreciation of the onset/end of the rainy season between farmers and technicians due to the occurrence of false starts/early cessation of the cropping period these last two decades (Alhassane et al., 2013). These perceptions might be strongly influenced by factors such as limited capacity to record climatic trends over a longer period of time, media and public opinion. In addition, the large number of illiterate farmers and the high spatio-temporal variability of the rainfall patterns could limit their memory capacity to remember the events over the years. Many studies on the perceptions of farmers with respect to climate change repeatedly did not show evident agreement between climatic data observations and the perception of farmers

on the onset and the cessation of rainy season (Mulenga & Ayala, 2014; Moyo et al., 2012; Simelton et al., 2013; Amadou, Villamor, Attua, & Seydou, 2015; Nyanga, Johnsen, Aune, & Kalinda, 2011). Thus, the perceptions of farmers should not be the only criteria when identifying gaps and needs of farmers, but rather criteria to be exposed to the objective facts which will enable them to take adequate adaptation measures.

The average annual temperature trends (minimum, maximum and mean) exhibited a positive trend in both districts suggesting a significant increase over the 20 years period. This was in agreement with respondents perceptions (84% and 81% respectively in Diffa and Aguié). These findings were in accordance with studies carried out by Mulenga and Ayala (2014). They found more consistency among observations related to temperature, with statistical evidence corroborating accounts of rising temperatures in all study sites in Zambia. Furthermore other studies (Ishaya & Abaja, 2008; Deressa et al., 2009; Gbetibouo, 2009; Fosu-Mensah, Vlek, & Manschadi, 2010; Shashidahra & Reddy, 2012) also indicated an increase in temperature over the years, resulting in increased frequency of drought. In this study, temperature remains the only parameter which the meteorological trend was clearly in agreement with the perceptions of farmers in both districts.

From the analysis of meteorological data, there is no evidence of any statistical significance on changes in rainfall patterns in both districts over the last 20 years. Nevertheless, based on their subjective observations, farmers expressed some coping strategies to adapt to climate change in their areas. A large percentage of the householders in Diffa (76 %) and in Aguié (54%)

adopted crop diversification by practicing mixed cropping. Crop diversification was also the most predominant adaptation strategy in farmers with less or more than 20 years farming experience. Hence, it is not evident whether this strategy is a response to the climatic change or is a traditional measure to reduce risk of crop failure due to climatic or other factors (e.g. pests). In fact according to farmers, mix cropping millet or sorghum with cowpea and vegetables for example allows them to harvest cowpea grains and vegetables when the growing season is too short for millet to grow successfully or when millet spikes get damaged by birds or insects.

Therefore, this strategy seems to be rather a traditional strategy to reduce crop risk failure in these agro-ecological zones than a specific response to climate change. It is well known amongst farmers that greater crop diversity and mixed cropping offer considerable protection against farming failure, including climate-related risk (Dejene, Midgley, Marake, & Ramasamy, 2011).

Nevertheless, other strategies (different planting dates, planting trees, use of organic fertilizers) were increasingly observed especially with young farmers (with <20 years farming experience). These strategies are reported as reliable solutions to climate change (Ibrahim, & Alex, 2008, Sarr et al., 2014). Thus, young farmers have seemingly more potential to diversify adaptation strategies to climate change. This could be due to their open mindedness towards the adoption of new technologies. The lack of knowledge of appropriate adaptation measures among the majority of farmers might be due to lack of suitable information on climate adaptation strategies and of the

means to invest in systems of improved agricultural technology. For many years in Niger, farmers have had to face harsh agro-pastoral environment leading to impoverishment. It will be difficult for them to sustain in addition the effect of climate changes. Farmers are attempting to adapt to on-going rainfall variabilities, but there were some constraints hindering them in this. These include lack of information, irregularity of extension agents visits, lack of subsidies and access to improved crop varieties, poor government attention to climate problems and low awareness level. However, barriers to adaptation strategies varied within districts.

For example the lack of information is the most important barrier to adaptation in Diffa, while in Aguié, lack of access to improved crop varieties is the main barriers. These results indicated in both locations a low access by farmers to extension services. Since 1998, the extension service has gradually declined and today there is no longer a working public agricultural extension services in Niger. Only the private sector and some NGOs played some role in providing extension and advisory services to the farmers. It is known that agricultural extension service is mainly responsible for awareness creation among farmers and has a strong reliance to exchange information among farmers (Hedjazi et al., 2006).

A number of studies (Mmbengwa, 2009; Anaeto et al., 2012; Mmbengwa et al., 2012; Ayanwuyi, 2013) have also underlined the important role of extension services in supporting farmers to adapt to climate change. According to IPCC (2011), climate change and its associated uncertainties implies that extension services need to regularly access new knowledge and

extend it in an adequate and timely manner to the farmers. Therefore, in Niger, where agricultural production remains the main source of income for most rural householders (86%), the re-implementation of the agricultural extension service should be a priority since it will significantly increase farmers' awareness of changing climatic conditions and effective adaptation measures.

## CHAPTER SIX

### SUMMARY, CONCLUSIONS, RECOMMENDATIONS AND FUTURE WORK

#### Summary

Surveys conducted in irrigated production zones of Niger revealed *Meloidogyne* spp and a wilt *Fusarium* sp. as the economically important pathogens of vegetable crops. Recently, infected plants by these pathogens are developing sudden death syndrome that leads to heavy production losses. Therefore the pathogens are becoming a significant threat to sweet pepper crop production in Diffa and Maradi and hence putting food security of the farmers at risk. This study was undertaken to assess the causal agents of the disease, their genetic diversity, and population structure, to understand how climate change (increase temperature) contribute to the development of the sudden death disease and the farmer's strategy adaptation. This information is primordial to design control methods for the prevention and control of the pathogens.

The first hypothesis was that the sudden wilt disease of sweet pepper was caused joint infection of *Meloidogyne* and *Fusarium* spp. In this study diversity of pathogens were identified in sweet pepper. PCR primers embedded in the COII and 16S genes produced a product size of 705-bp and 1.6 kb respectively for *M. enterolobii* and *M. incognita*. The digestion with Hinf I of 1.6-kb product of *M. incognita* yielded two fragments of 1.23 and 0.37. The primers Me-F/Me-R yielded 200-bp which are specific to M.

*enterolobii*. The study also clearly indicates that *M. enterolobii* is established in Niger and would be dramatically attacking the economically important crops. PCR amplification of *Fusarium spp* with ITS1/ITS4 and EF1/EF2 yielded respectively in 570 and 700-bp band and revealed that all sequences shared 99% identity with *Fusarium solani*.

The second hypothesis was that the populations of pathogens are influenced by local practices and climate variability. The study clearly indicated a high incidence of *Meloidogyne* and frequency of *Fusarium*. The incidence of the *Meloidogyne spp* was 100% in both districts while the occurrence of *Fusarium spp* was 80.32% in Diffa and 62.23% in Aguié. The monocultures in sandy soil with susceptible sweet pepper varieties in the study area are factors that contributed to the high reproduction of pathogens.

The third hypothesis of the study was to explore and test the hypothesis that the sudden death syndrome of sweet pepper is caused by co-infection of *M. enterolobii* and *Fusarium solani* and this co-infection is influenced by the temperature. Single and combined inoculation of the nematode and fungus was done to observe the disease severity in the plants due to the individual effect and interactive effect of nematode and fungus. The results in this experiment were not being able to cause the sudden death of sweet pepper plants with single and combined inoculation of the nematode and fungus.

### **Conclusions**

During 2013-2014, a survey was carried out in two sweet pepper growing sites, Diffa and Aguié and investigated the perception of farmers on

climate variabilities, adaptation strategies to climate and their access to agricultural extensions services. In the other hand, this study examined the presence and identification of *Meloidogyne* spp. and *Fusarium* spp. The co-infection of *Meloidogyne enterolobii* and *Fusarium solani* was also examined. From the major findings of this study, these conclusions are made:

- The results indicated that the incidence of *Meloidogyne* spp. was 100% at all sites whereas the incidence of *Fusarium* wilt was 30%. The nematode root population densities and frequency of *Fusarium* also were high at both Diffa and Aguié.
- In this study, 10 genera of plant parasitic nematodes were identified and the genus *Meloidogyne* was the most predominant at all sites. Three pathogens were identified as causal agent of wilt disease in sweet pepper: *Meloidogyne enterolobii*, *Meloidogyne incognita* and *Fusarium solani*. Two were for the first time recorded on sweet pepper plants in Niger (*M. enterolobii* and *F. solani*).
- In the controlled experiments, the co-infections of *M. enterolobii* and *F. solani* sweet pepper did not cause sudden death disease syndrome as observed in the field.
- Temperature significantly influences the reproduction capacity of pathogens and hence their abundance and frequency of occurrence. The optimum temperature for abundance and frequency of *Meloidogyne* and *Fusarium* is between 23-27°C. At 30°C, abundance and frequency of both *Meloidogyne* and *Fusarium* significantly reduces.



- The study provides insight into the perceptions and adaptations to climate variabilities in two agro-ecological zones Diffa and Aguié in Niger Republic. The perception of farmers on rainfall patterns do not match with past meteorological records but their perception in change of temperature are in line with increase temperature over the past 20 years. The study revealed also that farmers have poor capacity to adapt to the climate variability due to the lack of extension services support.

### **Recommendations**

Based on the findings of this study, the following recommendations are made:

1. The causal agents of wilt disease in Diffa and Aguié were *M. enterolobii* and *F. solani*. Aggressive nature of *M. enterolobii* could have contributed to the 100% incidence and susceptible nature of varieties of sweet pepper being cultivated. It would be advisable to breed for new varieties that may be resistant to *M. enterolobii* and *F. solani*.
2. The practice of using farmers' saved seeds for cultivation must be stopped, as *F. solani* is seedborne. It may be necessary to obtain certified seeds or treated seeds for planting.
3. The practices of monoculture with susceptible sweet pepper plants increase the RKN infestation. Cultivation of sweet pepper in such way should be avoided, and rotation of non-host crops may serve to reduce the crop damage.

4. High Temperatures prevail in Diffa and Aguié and since temperature reduces the abundance of *M. enterolobii* and the frequency of occurrence of *F. solani*, soil solarization could be used as one of the adaptation strategies in managing *M. enterolobii* and *F. solani* as well as weed seeds.
5. Build knowledge of farmers about climate impacts and management of disease through field schools

#### **Future work**

- Investigate the extent of occurrence or distribution and spread of *M. enterolobii* and *F. solani* over Niger
- Test the effect of the two pathogens (*M. enterolobii* and *F. solani*) at various inoculum levels under different temperature and water regimes on the development of the wilt syndrome in the field
- Identify climatic and biotic factors that trigger the development of sudden death syndrome of sweet pepper plants

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## APPENDICES

### Appendix A- Anova for the effect of treatment on *Meloidogyne* abundance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	1.02E+08	20485834	6.377	6.88e-05 ***
Residuals	66	2.12E+08	3212407		

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

### Appendix B- Anova for the effect of treatment on *Fusarium* frequency

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	56072	11214	11.55	4.93e-08 ***
Residuals	66	64067	971		

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

### Appendix C- Anova for the effect of treatment on the yellowing leaves

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	2198	439.6	6.377	0.000621 ***
Residuals	66	5820	88.2		

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

### Appendix D- Anova for the effect of treatment on the total leaves

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	453	90.59	0.381	0.00086 ***
Residuals	66	15713	238.07		

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Appendix E-** Anova type III of the effect of temperature and infection of sweet pepper plants by *Meloidogyne* or *Fusarium* on *Meloidogyne* abundance.

	Df	Sum of Sq	RSS	F value	Pr(>F)
<none>	607057	686.42			
Temperatures	1	21103433	81809216	14.6007	0.000433***
I(Temperatures^2)	1	25150037	85855820	17.4003	0.000149***
Inoc <i>Fusarium</i>	1	8177	60713960	0.0057	0.9404
<i>Meloidogyne</i> first	1	1642220	62348003	1.1362	0.292548
<i>Fusarium</i> First	1	838882	61544665	0.5804	0.450416

**Appendix F-** Anova type III for the effect of temperature and sweet pepper plant infection by *Meloidogyne* or *Fusarium* on *Fusarium* frequency.

	Df	Sum of Sq	RSS	F value	Pr(>F)
<none>	4458	229.5			
Temperatures	1	21890.7	26348.7	206.2392	<2e-16 ***
I(Temperatures^2)	1	24045.4	28503.3	226.5387	<2e-16 ***
inoc <i>Fusarium</i>	1	4	4462	0.0377	0.847
<i>Meloidogyne</i> first	1	122.4	4580.4	1.1532	0.289
<i>Fusarium</i> First	1	2.7	4460.7	0.0258	0.8733

**Appendix G-** Anova type III for the interaction between *Meloidogyne* and *Fusarium* (only with data with both inoculations)

	Df	Sum of Sq	RSS	F value	Pr(>F)
<none>	6463.1	194.85			
J2skg	1	7449.8	13913	36.885	8.780e-07 ***
Temperatures	1	10844.4	17307.5	53.692	2.486e-08 ***
J2skg:Temperatures	1	9187.6	15650.7	45.489	1.281e-07 ***

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

**Appendix H- Household questionnaire: Farmer's perception of climate change, adaptations and access to agricultural extension services**

**Farmer personal and confidential data**

Name of farmer .....

Address of farmer.....

Age .....

Sex .....Male  Female

Telephone .....

Surveyed zone.....

Name of interviewer .....

Telephone .....

Date of interview (MM/DD/YYYY)...../...../.....

**Variability climatic**

1. Where have you heard about climate change

- Self-feeling
- TV
- Radio
- Newspapers
- Relatives
- Government agencies/  
information

2. What are the indicators of onset agricultural season?.....

3. What are the indicators of end of agricultural season.....

4. What are the indicators of good agricultural season.....  
.....

5. What are the indicators of bad agricultural season.....  
.....

6. Have you noticed any change in rain onset over the 20 past years?

Early



No change

Late

7. Have you noticed any change in rain cessation?

Early

Constant

Late

8. Have you noticed any change in the length of the rainy season?

Increased

Decreased

Constant

9. Have you noticed any change in temperature?

Increased

Constant

Decreased

#### **Measures for climate adaptation**

10. What methods are you using to adapt to climate change?

Crop diversification

Different planting date

Mulching & planting trees

Changing fertilizers

Soil conservation

Others adaptations

No adaptation

#### **Constraints to climate change adaptation**

11. What are the constraints you face in adapting to climate change?

Lack of information

Irregularity of extension services

No subsidies

Lack of access to improved crop varieties

Poor government attention to climate problems

Low awareness level

#### **Social characteristics of farmer**

12. How old is the farmer?

25 to 29

29 to 30

30 to 39

- 39 to 40
- 40 to 49
- 49 to 59
- 59 to 69
- $\geq 70$

13. What is farmer's farming

Experience (years)

- $\leq 10$
- 11 – 20
- 21 – 30
- 31 – 40
- $\geq 41$

14. What is farmer's educational level?

- No formal education
- Primary education
- Secondary education
- Tertiary education

15. Have you been trained on Sweet peppers diseases and control methods?

- yes  no

16. Do you receive the visit extension workers?

- Never
- Rarely
- frequently

## **Appendix I: LIST OF PUBLICATIONS**

- Assoumana, B. T., Ndiaye, M., Puije, G. C. V. D., Diourte, M., & Gaiser, T. (2016). Comparative Assessment of Local Farmers' Perceptions of Meteorological Events and Adaptations Strategies: Two Case Studies in Niger Republic. *Journal of Sustainable Development*, 9(3), 118-135.
- Assoumana, B. T., Samer, S. B., Ndiaye, M., Puije, G. C. V. D., Sarr, E., Haougui, A., Grundler, F. M., Elashry, A. (2016). Morphological and molecular identification of *Meloidogyne enterolobii* infesting sweet pepper in southern and eastern Niger. **Submitted to *Plant Disease*.**
- Assoumana, B. T., Ndiaye, M., Puije, G. C. V. D., Grundler, F. M. (2016). Interactive effect of co-infection of *Meloidogyne enterolobii* and *Fusarium solani* on the severity of sudden wilt of sweet pepper plants in Niger. **To be submitted**
- Assoumana, B. T., Ndiaye, M., Puije, G. C. V. D., Elashry, A. (2016). Morphological and molecular identification of *Fusarium solani* infesting sweet pepper in Niger. **To be submitted**