ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 8, Issue 3, pp: (1-9), Month: July - September 2021, Available at: www.paperpublications.org

Identification of Newly Emerging Rice Diseases in Fogera Plains, Ethiopia

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Abstract: **Rice was introduced in to Ethiopia around 1970s based on the discovery of wild rice in Fogera and Gambela plains. Since then, it is widely cultivated in the country especially in Fogera plains, and becomes a popular crop in the area. However, in Fogera plains, complexes of unknown and newly emerged diseases are being observed both in the farmers' fields and research station and seriously affects the productivity of the crop. Accordingly, field assessment and laboratory works were conducted with the objective to identify newly emerging rice diseases and their causal organisms in Fogera plains, Ethiopia. A total of 46 samples (17soil, 10 root and 19 foliar) were collected systematically from infected rice fields in the three main rice growing districts (Fogera, Dera and Libokemkem) and 73sub samples were examined for the possible pathogens at Ambo Agricultural Research Center Laboratory. Out of 46 samples and 73 subsamples examined for the three pathogens, 28 samples were infected by different pathogens. Of which 16 samples by bacteria (Xanthomonas spp. and Pseudomonas spp.), nine by fungal (Pythium spp., Sarocladium spp. and Fusariu spp.), three by nematode (Hoplolaimus spp., Tylenchorhynchus spp. and Helicotylenchus spp.). Most samples were infected by more than one pathogen and the resulted disease was complex. Moreover, the identified pathogens' genera were new to the area. Therefore, proper pathogen identification should be done further up to the species level and the real disease causal organism should be identified following Koch's postulate.**

Keywords: **emerging diseases, rice diseases, identification, diseases complex, rice, Fogera plain.**

1. INTRODUCTION

Background and Justification

Rice (*Oryza sativa* L.) is the major caloric source for a large portion of the earth's population (Smith and Dilday, 2003). However, this crop can seriously be affected by a large range of diseases which can destroy up to 40% of the world's rice crops (Oerke, 2006). Rice is attacked by more than 76 diseases caused by fungi, bacteria, viruses, mycoplasma like organisms and nematodes (Hafiz et al., 2009). Moreover, rice can be attacked by one or more pathogens at any stage, starting from germination up to physiological maturity (Claudius-Cole, 2018). The diseases range from foliar diseases such as brown leaf spot, rice blast, bacterial blight to root diseases caused by Pythium, Fusarium and nematodes (Ou, 1985). These diseases could be either seed born, soil born, air born or a combination of all the three and can spread to a new area through their carriers, finally causing newly emerged diseases to that area.

An emerging disease is a case or group of cases that are newly recognized or newly appeared in an area and can increase fast in incidence and severity (Daszak et al., 2003). Emerging plant pathogens can be devastating for cultivated crops; hence, they have been included in global lists as quarantined pathogens (Avila-Quezada et al., 2018). Moreover, when a pathogen is introduced to a new area, it is likely to be serious to infect its host. This is because of that, since the crop and the pathogen were not coevolved, the crop is not adapted the pathogen or not develop resistance mechanism against the

ISSN 2349-7823

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pathogen. This problem is a major challenge in areas which are not center of origin for some crops and absence of wild relatives, hence introduce planting materials from abroad, like rice in the case of Ethiopia.

Rice was introduced in to Ethiopia around 1970s based on the discovery of wild rice in Fogera and Gambela plains (Gebey et al., 2012). Now a day, it is widely cultivated in Fogera plains mostly by introducing germplasms from abroad. However, complexes of unknown and newly emerged diseases are being observed in the area and threaten the rice production. Moreover, in Ethiopia, diseases and unavailability of management options are ranked as the second constraint of rice production and productivity (NRDSE, 2009). Due to this, the national average productivity of rice in Ethiopia is 2.888 tone /ha (CSA, 2018), which is low as compared with the world average productivity 4.66 tone/ha (FAOSTAT, 2021).

Thus, there is a need to tackle the diseases problem through appropriate management measures so as to avoid yield loss. To do so, identifying the true causal pathogen and apply corresponding management option is strategy (Agrios, 2005). Hence the present study was carried out with the objective of identifying newly emerging rice diseases in Fogera plains, Ethiopia.

2. MATERIALS AND METHODS

Location

The survey was conducted in the three major rice growing districts (Fogera, Dera and Libo kemkem) of Fogera plains. A total of seven kebeles and 23 fields were assessed approximately within 1-5km distance using the cars odometer based on the presence of rice field and road accessibility (figure1). The kebeles were selected purposely based on their rice growing potential.

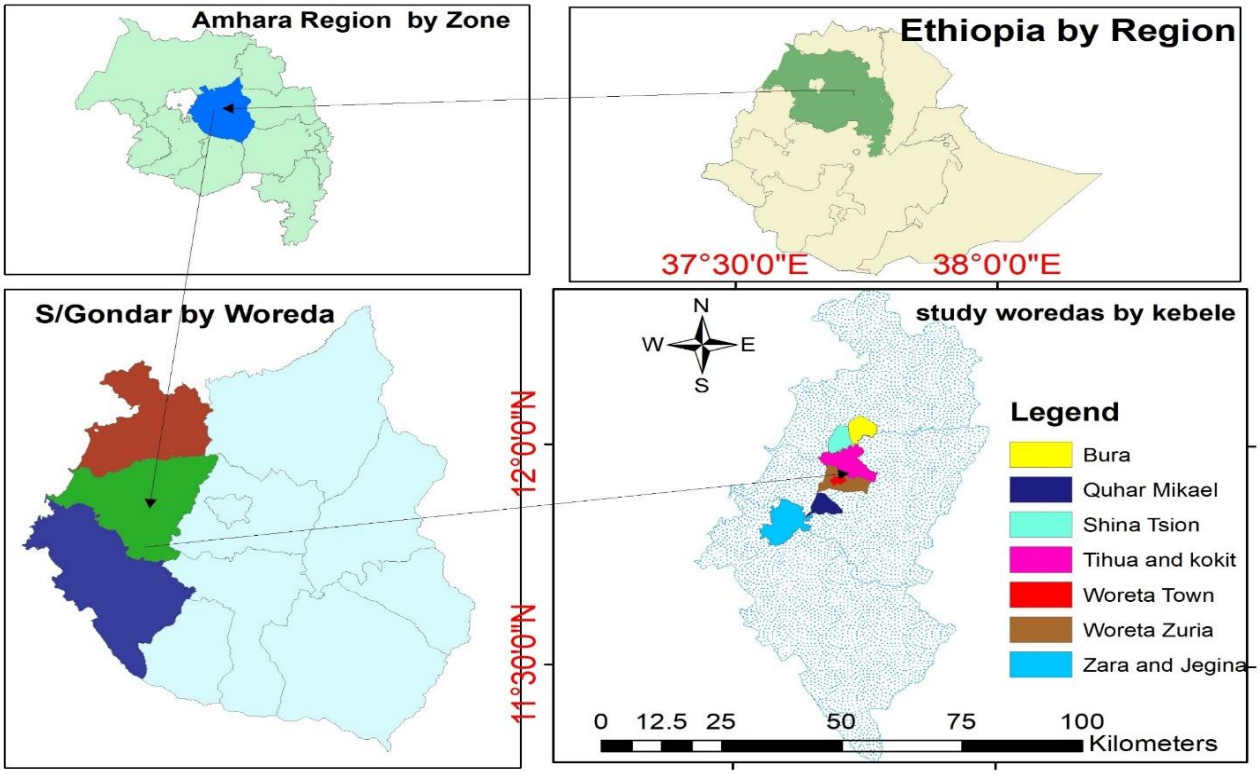

Figure 1. Map of study area

Sample collection

A systematic survey of the newly emerged disease complexes of rice was conducted in 2019 main cropping season. Samples were collected systematically by observing diseases symptoms in rice fields which are suspected to be infected with newly emerging diseases causal pathogen/s. Both plant and soil samples were collected at each point in the infected

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rice fields following an **'X'** shape fashion at an approximate distance of 1-5km in each assessed field. A total of 46 samples (17 soil, 10 root and 19 foliar) were collected. GPS, Auger, Scissors, Ice box, Plastic bag, Paper bag, pen, paper and pencil were used to collect, record, label, and transport samples (fig. 2).

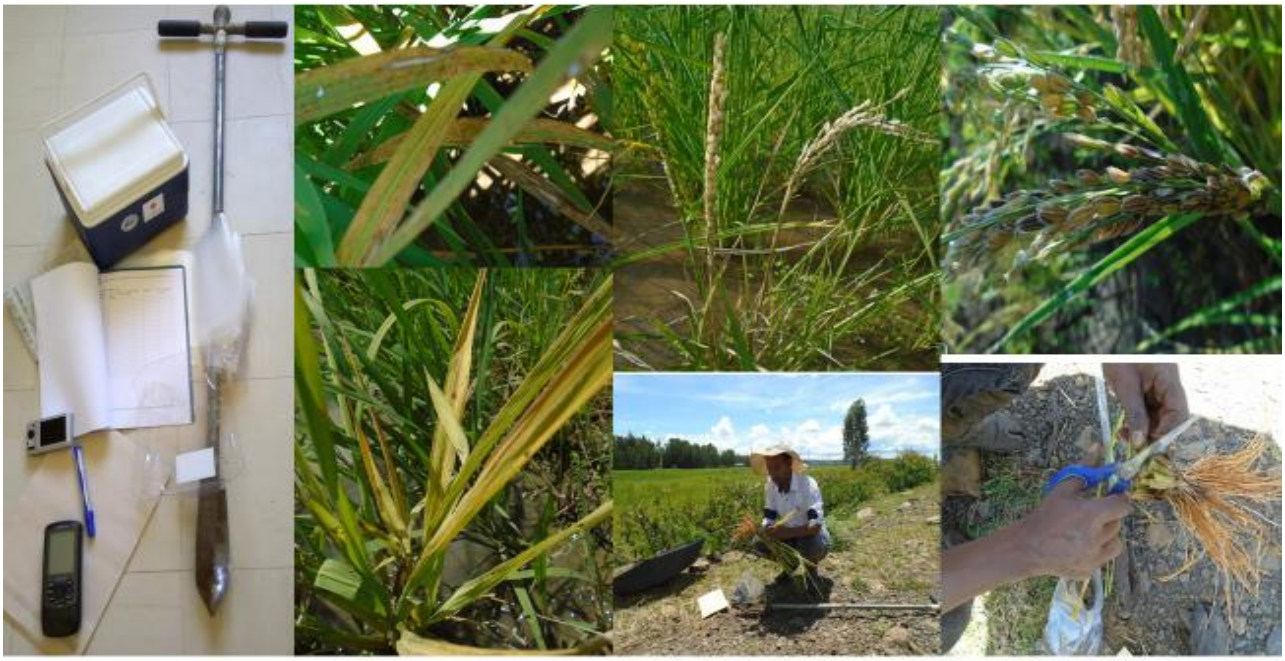

Figure 2. Sample collection and materials used during sample collection

During the sample collection process; GPS coordination of the field, soil type and cropping history of the field, ecology of the field (upland or lowland) and all other necessary information related to the samples and the fields were recorded and tagged for each sample. The collected samples were kept in refrigerator for two days until taken to Ambo ARC laboratory for identification.

Identification

The collected samples were taken to Ambo Agricultural Research Center laboratory for identification. Samples were examined for the three possible disease causal pathogens (Fungi, Bacteria and Nematode) based on the standard procedures (Burgess et al. 2008). Root and foliar samples were examined for fungal and bacterial pathogens while soil and root samples were examined for nematode pathogens.

All types of pathogens associated with the samples were identified morphologically following the standard procedures and specific/appropriate identification processes for each of the three pathogen types (Barnett and Hunter, no date; Goszczynskk et al., 2000; Burgess et al., 2008; Coyne et al., 2014).

I. Identification of fungal pathogens

A total of 26 samples (nine root and 17 foliar) were examined for fungal pathogens. Samples were surface disinfected from saprophytes with 70% ethanol alcohol for three minutes and rinsed with sterilized distilled water three times. Pieces of surface disinfected samples were cultured on PDA and incubated at room temperature for seven days until spore development. After sporulation, contaminated and mixed cultures were sub cultured to obtain the pure culture of the pathogen. Then, according to Agrios (2005), the mycelia shape, size, color, growth and manner of arrangement of spores on the sporophores or in the fruiting bodies were used to distinguish the fungal genera (Figure 3).

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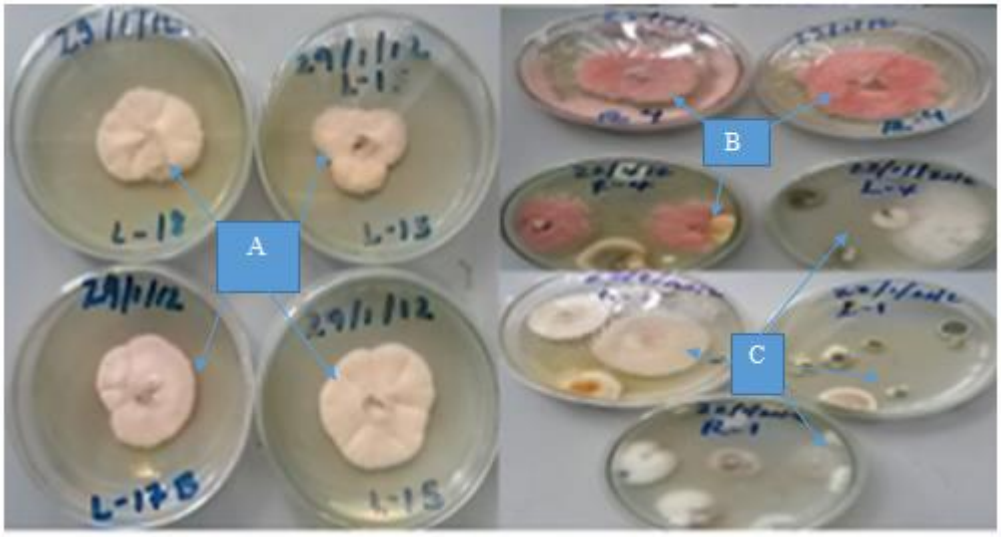

Fig.3. Pathogenic fungal genera; A) *Sarocladium* spp., B) *Fusarium* spp., C) *Pythium* spp.

II. Identification of bacterial pathogens

A total of 22 samples (7 root and 15 foliar) were examined for bacterial pathogens. Samples were surface disinfected from saprophytes with 70% ethanol alcohol for three minutes and rinsed with sterilized distilled water three times. These surface disinfected samples were macerated using sterilized pestle and mortar with the addition of drops of sterilized distilled water to get crude juice and then streaked on nutrient agar media using inoculation loop and incubated at room temperature for two days. Finally, different biochemical and physiological tests were conducted (Fig. 4) to identify the bacteria genera type (Goszczynskk et al., 2000).

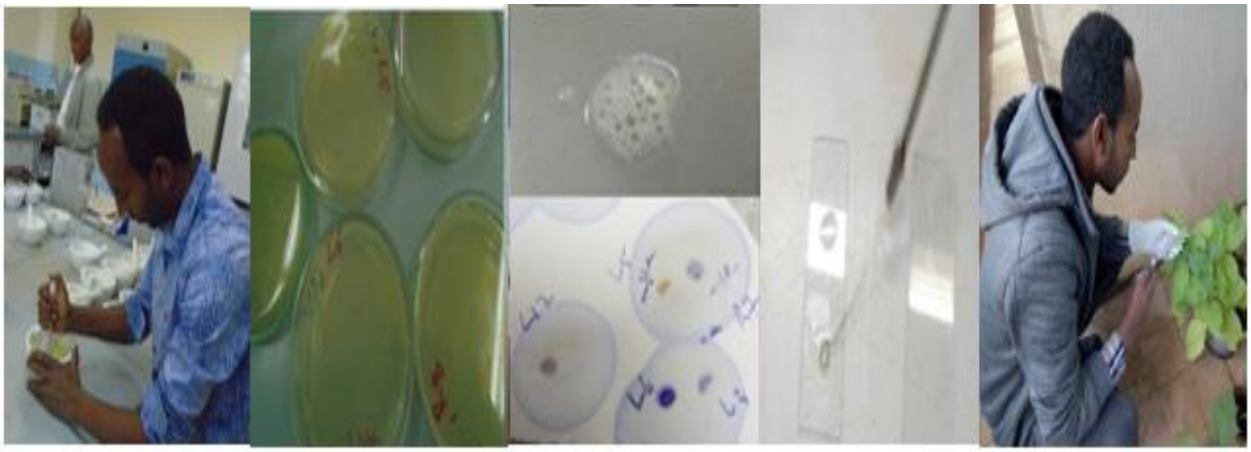

Fig.4. Plant pathogenic bacteria genera identification procedures and different tests

III. Nematode identification

Soil and root samples were examined for the presence of plant parasitic nematodes. A total of 25 samples (1

7 soil and 8 root) were examined following series of standard procedures as follows (Coyne et al., 2014).

Debris and lumps were removed from the soil by coarsely sieving the sample. The roots were washed gently in tap water and chopped. Standard sized sub-samples, i.e., 100 ml soil (by volume) and 20 g chopped root were measured out. Then, sieves with tissue paper were lined and placed on plastic plates. The samples were placed on the tissue by ensuring that they remain on the tissue and do not to spill over the edges. The water was poured into the tray carefully down the gap between the tray and the sieve, and keeping it for two to three days remaining wet to allow the nematodes to drain from the sample to the water. Then the water was drain carefully while the sieves were removed from the trays and the tissue paper. Soil and the root were then discarded. The water containing the nematodes was poured into a labeled beaker and

ISSN 2349-7823

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the trays were thoroughly rinsed into the beaker. Finally, the samples in beakers ready for assessing nematodes were examined under compound microscope for the presence of nematodes in similar way for each sample (fig. 5).

Fig.5. Nematode identification

Results and Discussion

The laboratory results indicated that, 69.57% of the assessed fields were infected by different diseases causal agents. Of which, 30. 43% of fields were infected by the complex of both fungal and bacterial pathogens while one field was also infected by the complexity of all the three (fungi, bacteria and nematode) pathogens (Table 1). This indicated that the rice fields in the assessed area are being infected not only by the newly emerging diseases but also by the complex disease causal agents. As indicated in figure 6 below, although the symptoms seem similar, the diseases and the diseases causal organisms are different. This condition makes the symptom-based identification more ambiguous (AICAF, 2001).

Moreover, out of the total of 46 collected samples and 73 examined subsamples, 28 (60.87%) of samples were infected by different pathogens viz.: sixteen samples by two bacteria genera (11by*Xanthomonas* spp. and 5by *Pseudomonas* spp.), nine by three fungal genera (four by *Pythium* spp., three by *Fusariu* spp. and two by *Sarocladium* spp.), three samples by three nematode genera (one by *Hoplolaimus* spp., One by *Tylenchorhynchus* spp. and *Helicotylenchus* spp.) (Table 1).

Table 1. Type of pathogens identified from infected rice plant and soil samples

Location	Field number	Sample codes	Identified pathogen genera under the three causal agents		
			Fungi	Bacteria	Nematode
On-station	1	L1	-	-	-
On-station	2	L2	-	*Xanthomonas* spp.	-
On-station	3	L3	*Fusariu* spp.	*Pseudomonas* spp.	-
On-station	4	L4	-	*Xanthomonas* spp.	-
On-station	5	L5	*Pythium* spp.	*Xanthomonas* spp.	-
		S1	-	-	-
On-station	6	L6	*Pythium* spp.	*Pseudomonas* spp.	-
		S2	-	-	-
On-station	7	L7	*Pythium* spp.	-	-
		S3	-	-	-
On-station	8	L8	-	*Xanthomonas* spp.	-
		S4	-	-	-
		R1	*Pythium* spp.	*Pseudomonas* spp.	-
Bura	9	L9	-	-	-
		S5	-	-	*Hoplolaimus* spp.
		R2	-	-	-
Bura	10	L10	-	-	-
		S6	-	-	-
Bura	11	L11	-	-	-
Shina Tsion	12	L12	-	*Xanthomonas* spp.	-

Shina Tsion	13	L13	*Sarocladium* spp.	*Xanthomonas* spp.	-
		S7	-	-	-
Shina Tsion	14	L14	-	-	-
		S8	-	-	-
		R3	-	-	-
Tihua zakana	15	L15	-	-	-
		S9	-	-	*Tylenchorhynchus* spp
		R4	*Fusarium* spp.	*Xanthomonas* spp.	-
Zara	16	L16	-	-	-
		S10	-	-	-
		R5	-	-	-
Zara	17	L17	*Sarocladium* spp.	*Xanthomonas* spp.	
		S11	-	-	-
		R6	-	*Pseudomonas* spp.	-
Jigna	18	L18	-	-	-
		S12	-	-	-
		R7	-	*Pseudomonas* spp.	-
Q/Michael	19	L19	-	-	-
		S13	-	-	-
		R8	*Fusarium* spp.	*Xanthomonas* spp.	
On-station	20	L20	-	*Xanthomonas* spp.	-
		S15	-	-	-
		R9	-	*Xanthomonas* spp.	-
On-station	21	S16	-	-	-
On-station	22	S17	-	-	-
On-station	23	S18	-	-	*Helicotylenchus* spp.

Fig.6. Multiple pathogens causing complex disease symptom and identified from samples taken on a single spot/stop of rice field.

Furthermore, as presented in the above (Table 1), out of the 28 infected samples, seven were infected by both bacteria and fungi. In addition, different samples collected from one field resulted more than one disease causal agents and/or even more than one genus of a single type of causal agent/pathogen. Moreover, most of the identified pathogens are new to the area.

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Out of the three fungi genera identified from nine samples (Table 2), only *Sarocladium* spp. (*Sarocladium oryzae*) was reported so far in Fogera plains (Tekalign et al., 2019; Desalegn et al., 2020). On the other hand, the two fungi genera (*Fusariu* spp. *and Pythium* spp.) were not reported, hence they are newly emerging in the area.

Table 2. Fungal pathogens identified from infected rice plant samples

Field number	Sample code	Location /kebelle	Type of fungal genera identified	Information in Fogera plain so far
3	L3	On-station	Fusariu spp.	New
5	L5	On-station	Pythium spp.	New
6	L6	On-station	Pythium spp.	New
7	L7	On-station	Pythium spp.	New
8	R1	On-station	Pythium spp.	New
13	L13	Shina Tsion	Sarocladium spp.	Reported
15	R4	Tihua zakana	Fusariu spp.	New
17	L17	Zara	Sarocladium spp.	Reported
19	R8	Q/Michael	Fusariu spp.	New

To characterize bacterial isolates, different morphological, biochemical and physiological tests such as colony color, shape, margin, texture, elevation, cell shape, KOH solubility, gram-negative and gram-positive tests, motility, pathogenicity/hypersensitive reactions on indicator plants were done following procedures indicated by Goszczynskk et al. (2000). Based on the characteristics, two groups/genera of bacteria (*Xanthomonas* spp. and *Pseudomonas* spp.) were identified from 16 samples (table 3).

Table 3. Bacterial genera identified from infected rice plant samples and their characteristics

Isolate/ sample code	Cell shape	Colony color on NA	Margin	Elevation	Texture	Colony shape	Oxidase	Catalase	KOH	Hyper. test on datura	Lac. Fermentation	Motility	Genus/group
L2	Rod	yellow	Round	Raised	Smooth	Round	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
L3	Rod	Cream	Round	Convex	Smooth	Round	+ve	+ve	+ve	+ve	-ve	+ve	*Pseudomonas* spp.
L4	Rod	Creamy	Round	Raised	Smooth	Round	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
L5	Rod	yellow	Round	Convex	Smooth	Round	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
L6	Rod	yellow	Round	Convex	Smooth	Round	+ve	+ve	+ve	+ve	-ve	+ve	*Pseudomonas* spp.
L8	Rod	Creamy	Round	Raised	Smooth	Round	W+ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
L12	Rod	Creamy	Round	Raised	Smooth	Round	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
L13	Rod	Creamy	Round	Convex	Smooth	Round	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
L17	Rod	Yellow	Round	Raised	Smooth	Round	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
L20	Rod	Creamy	Round	Convex	Smooth	Round	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
R1	Rod	Creamy	Round	Convex	Smooth	Round	+ve	+ve	+ve	+ve	-ve	+ve	*Pseudomonas* spp.
R4	Rod	creamy	Round	Convex	Smooth	Round	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
R6	Rod	Creamy	Round	Convex	Smooth	Round	+ve	+ve	+ve	+ve	-ve	+ve	*Pseudomonas* spp.
R7	Rod	Creamy	Round	Convex	Smooth	Round	+ve	+ve	+ve	+ve	-ve	+ve	*Pseudomonas* spp.
R8	Rod	Cream	Round	convex	Smooth	Circular	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.
R9	Rod	Yellow	Round	Raised	Smooth	Circular	-ve	+ve	+ve	+ve	-ve	+ve	*Xanthomonas* spp.

Of the two types of bacteria group/genera, *Xanthomonas* spp. was dominant/frequently occurred on most samples. Moreover, according to Wasihun and Filagote (2016) and Tekalign et al. (2019) the *Xanthomonas* spp. was reported in different parts of Ethiopia in general and in Fogera plain and Pawe areas in particular and the *Pseudomonas* spp. is identified fer the first time in the study area (Table 4).

Table 4. Bacterial pathogens identified from infected rice plant samples

Field number	Sample code	Location	Genus/group	Information in Fogera plain so far
2	L2	On-station	*Xanthomonas* spp.	Reported
3	L3	On-station	*Pseudomonas* spp.	New
4	L4	On-station	*Xanthomonas* spp.	Reported
5	L5	On-station	*Xanthomonas* spp.	Reported
6	L6	On-station	*Pseudomonas* spp.	New
8	L8	On-station	*Xanthomonas* spp.	Reported
12	L12	Shina Tsion	*Xanthomonas* spp.	Reported
13	L13	Shina Tsion	*Xanthomonas* spp.	Reported
17	L17	Zara	*Xanthomonas* spp.	Reported
20	L20	On-station	*Xanthomonas* spp.	Reported
8	R1	On-station	*Pseudomonas* spp.	New
15	R4	Tihua zakana	*Xanthomonas* spp.	Reported
17	R6	Zara	*Pseudomonas* spp.	New
18	R7	Jigna	*Pseudomonas* spp.	New
19	R8	Q/Michael	*Xanthomonas* spp.	Reported
20	R9	On-station	*Xanthomonas* spp.	Reported

Similarly, all of the three nematode genera identified in our study are new/not reported so far in Fogera plain as well as in Ethiopia as presented in table 5 below.

Table 5. Plant parasitic nematode genera identified from soil samples in infected rice field

Field number	Sample code	Location /kebelle	Type of nematode genera identified	Information in Fogera plain so far
9	S5	Bura	Hoplolaimus spp.	New
15	S9	Tihua zakana	Tylenchorhynchus spp.	New
23	S18	On-station	Helicotylenchus spp.	New

3. CONCLUSION AND RECOMMENDATIONS

A total of 23 fields were assessed in 2019 main cropping season to identify newly emerging rice diseases in Fogera plains. Of these: 14, 3 and 6 were assessed in Fogera, Dera and Libo kemkem districts, respectively. During the survey, 46 samples were collected and 73 subsamples were examined for the three pathogens. Out of 73, 28 samples were infected by different pathogens viz.: sixteen samples by two bacteria genera (11by*Xanthomonas* spp. and 5by *Pseudomonas* spp.), nine by three fungal genera (four by *Pythium* spp., three by *Fusariu* spp. and two by *Sarocladium* spp.), three samples by three nematode genera (one by *Hoplolaimus* spp., One by *Tylenchorhynchus* spp. and *Helicotylenchus* spp.)

Furthermore, most samples were infected by more than one pathogen and resulted diseases were complex. Generally, eight rice complex disease causal pathogens genera were identified. Moreover, out of the total eight identified pathogens' genera, six (*Pythium* spp., *Fusariu* spp., *Pseudomonas* spp., *Hoplolaimus* spp., *Tylenchorhynchus* spp. and *Helicotylenchus* spp.) were new to the area.

Therefore, to tackle the problem caused by these diseases complex caused by newly emerging pathogens:

- ☞ Proper pathogen identification should be done up to the species level
- ☞ The correct causal organism should be identified following Koch's postulate
- ☞ Appropriate management method should be developed accordingly the identified pathogens.

ACKNOWLEDGEMENT

We would like to acknowledge Ambo Agricultural Research Center plant pathology researchers and laboratory technicians.

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