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PATHOLOGICALASSESSMENT OF MAJOR FUNGAL PATHOGENS ASSOCIATED WITH TOMATO AND PEPPER IN JAMA'ARE NORTH-EASTERN NIGERIA

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ABSTRACT

Fungal diseases are the major threat and limiting factor in the production of economic crops which causes a tremendous loss of yield annually. The aim of this study was to assess the major fungal pathogens associated with tomato and pepper with a view to identify their presence incidence and severity. Field assessment was conducted in five irrigation villages in Jama'are during the dry season. At each location, 50 plants were assessed in fields by counting number of infected crops observed in an "X" pattern across the fields. Infected portion of diseased samples were collected for microscopic identification of the pathogens. Pathogenicity test was carried out to confirm the ability of the isolated pathogens to produce typical symptoms of the disease on the healthy plants. Data obtained were subjected for analysis of variance (ANOVA) at 5% level of significance. The result of the study shows that tomato recorded the highest disease incidence and severity in Dako-dako and DogonJeji while pepper recorded the lowest disease incidence and severity at Digiza. A total of 16 different fungal pathogens were identified based on cultural morphology and microscopic identification on potato dextrose agar (PDA) at 25±2ºC. Pathogenicity test revealed that 13 fungal pathogens were highly pathogenic to tomato and pepper. Identified fungal pathogens are the main causal agent of diseases in the study area which cause substantial losses and damages to the economic crops. It is recommended that an integrated approach involving proper agronomic practices should be applied for sustainable yield production in the study area.

Key words: Fungal, Incidence, Severity Pathogens, and Pathogenicity

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most popularly produced and extensively consumed vegetable crops in the world (Ayandiji *et. al*., 2011). Currently, more than 200 pests and diseases have been identified in tomato, causing losses in their production directly or indirectly (Alam *et al.,* 2007). Capsicum (*Capsicum spp*) is a main vegetable and spice crop originated in the American tropics and today cultivated all over the world for fresh, dried, and processing products. It has the highest content of vitamin C among all plants and has important medicinal properties such as prevention of heart disease, actuation of blood ambulation and antioxidant characteristics (Salehi, 2006).

Despite the importance of pepper and tomato as vegetables they are prone to a number of fungal diseases that reduce their marketability. The major fungal pathogens affecting tomato and pepper species include *Phytophthora capsici* a soil borne fungus that invades the base of the stem and causes a sudden and complete wilting of the aerial parts, especially when pepper and tomato are planted in furrow irrigation (Lema, *et al.*, 2018). Fungal diseases contribute both to the decrease in yields and to the deterioration of crop quality (Rozewicz*et al.*, 2021). In recent years, fungal diseases have occurred in many parts of the world and are considered to be one of the main factors affecting yield and yield quality (Nowicki *et. al.*, 2013).

It was reported by Bauchi State Agricultural and Rural Development Project (BARDP) in 2018/2019 that tomato and pepper are the most commercial vegetables grown in the area but due to the fungal infections the rate of production is reduced which leads to economic meltdown. Considering the importance of these crops as source of nourishment for a stable food security and a tool for income generation and for farmers well, it is important to assess the status of some of the fungal diseases associated with tomato and pepper. This research will serve as the baseline information on the effect of fungal diseases that causes tremendous loss of tomato and pepper. The research will also try to figure the main pathogens that limit and threatened tomato and pepper production in the study area.

MATERIALS AND METHODS

Study Area: The research was carried out at Dako-dako, Digiza, Dogon jeji, Gilar and Gongo located in Jama'are, Bauchi State, Nigeria lies between latitude 11°40N and longitude 9°53E, at a distance of 200 kilometers north eastern part of Bauchi State. These sites were chosen been among the largest sites producing tomato and pepper in Jama'are cultivation sites.

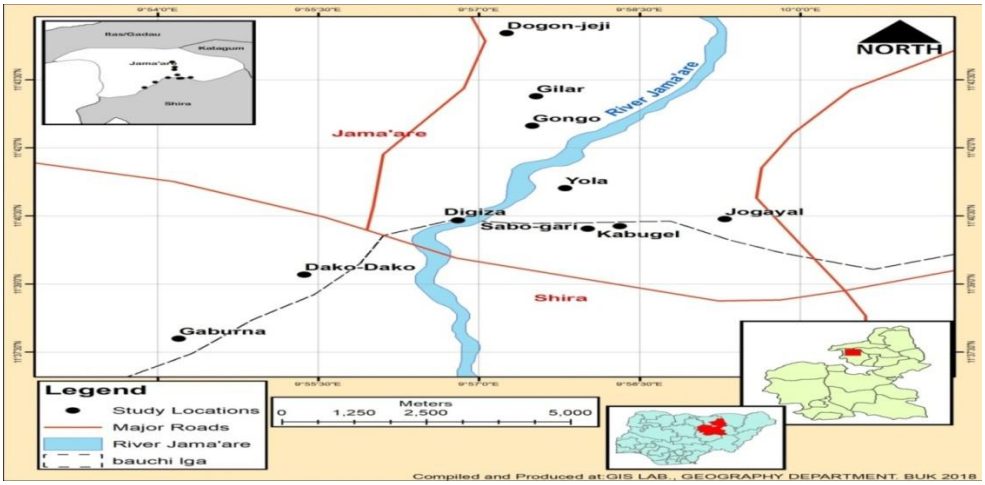


Figure 1: Location of Jama'are local govt. area, Bauchi state, Nigeria showing the study areas

Sampling technique: At each location (Dako-dako, Digiza, Dogon jeji, Gilar and Gongo) a 10m^2 plot was measured using 30-meter measuring tape, a total of 50 plants were selected using simple random sampling techniques obtained in an "X" pattern across each of the selected farm (Kutama*et al.*, 2007).

Data collection: Data were obtained at vegetative and reproductive growth stages for a period of three months at weekly bases. At each location, 50 crop plants were assessed in the farmers' fields by counting number of infected parts (stems, leaves and fruit) as described by Zarapi and Amechebe (2005) and Kutama *et al.* (2009). The presence of pathogens was determined by observing the infected plants using hand lens magnifying glass (Ngugi *et al.*, 2002).

Assessment of disease incidence and severity: Disease incidence was obtained by counting the number of infected plants out of the 50 plants observed at 7 days' interval (Kutama *et al.*, 2007). Percentage disease incidence was calculated by using the following formula:

$$\text{Percentage disease incidence (PDI)} = \frac{Total\ number\ of\ infected\ Plants}{Total\ number\ of\ Plants\ assessed}\ x100$$

The diseases severity was obtained by observing critical damages such as yellowing and dropping of leaf, wilting, vascular discoloration, root rots and fruits rots were considered as indices for determining disease severity using formula (Sethumadhava*et al.*, 2016).

$$\text{Severity} = \frac{sum\ of\ numerical\ ratings}{Total\ number\ of\ Plants\ assessed\ x\ maxium\ scored\ on\ scale}\ x100$$

The disease severity rating scale of 1–5 was based on critical damages caused by the fungal pathogens on leaves, stems and fruits (Sethumadhava*et al.*, 2016).

Isolation of fungal Pathogens: Infected parts of the plant were sampled aseptically and kept separately into polythene bags, preserved in ice box, and brought to laboratory for morphological examination and identification of fungal pathogens. Infected samples were washed with running tap water (Sethumadhava*et al.*, 2016). An appropriate size of diseases portion was carefully cut with the aid of sterile blade then sterilized with 70% ethanol for 30 and 60 seconds respectively and rinsed in sterile distilled water. Sliced portion were then plated on sterile PDA medium and 2% tetracycline was used to inhibit bacterial growth and then incubated at 25± 2 ^{0}Cfor 5-6 days (Sajad*et*

al., 2017). The colonies thus developed are repeatedly sub cultured on PDA medium to obtain pure cultures (Temesgen and Sefawdin, 2020).

Identification of fungal pathogens: The spores of fungi were taken aseptically, mounted on a slide, stained with lacto phenol cotton blue, and covered with a cover slip to examine under a microscope (40x) (Sajad*et al.,* 2017). Isolated fungi were identified based on colony color, number of septation, nature of hyphae (septate or aseptate) and shapes of spores (macroconidiaand microconidia) werecarefullyobserved using microscope (Temesgen and Sefawdin, 2020). The Morphological characteristics and appearance of the fungal pathogenwere confirmed and authenticated with the help of mycological atlas of common plant pathogenic fungi as described byUlhan *et al.,* 2006, (Wasanabe, 2010; Luz *et al.*, 2017).

Pathogenicity test: Healthy seedlings of tomato and pepper were collected from the farmland and seedlings were transplanted in polythene bags in an opened space containing 10 kg of sterilized soil. Three seedlings were used for each of the pathogen while control remained untreated with any of the pathogens. Conidial Suspension was prepared using 14 days' pure cultures in PDA. A sterile wire loop was used to scrape of the conidia and bring them to suspension. The suspension was filtered througha sieve to remove mycelial fragment andthen collected filtrate diluted serially to 1×10^5 ml^{-1}. A haemocytometer was used to adjust the spore concentration before inoculation (Wanjiku *et al.*, 2020).

Inoculation: Inoculation was carried out using injection method, the inoculums were introduced into the stem with a clean hypodermic needle, 1ml of the inoculum was injected to each plant (Catroux *et al.,* 2001; Kutama *et al.,* 2013). Foliar Inoculation was carried out, 5 ml was used to inoculate the leaves of the growing seedlings using spray pump (Kutama *et al.,* 2013). Inoculated plants were covered with polythene to ensure equal environmental condition and avoid contamination. The inoculated seedlings were observed after 21 days to determine the presence of necrotic symptoms in the aerial part. Subsequently, the pathogen was re-isolated to confirm the Koch's postulates (Paletto *et al.,* 2020). To determine the severity a visual scale was designed based on the percentage of infected plants, where 0= no visible rot; 1= 1–25% rot or chlorosis of leaves; 2= 25–50% rot or chlorosis of leaves; 3= 50–75% rot or chlorosis of leaves; 4= ≥75% rot or the major part of the plant wilted or death (Isaac *et al.,* 2018).

All data collected were subjected to Genstat statistical software 17th Edition for analysis of variance (ANOVA), where means were separated at 5% level of significance.

RESULT AND DISCUSSION

Percentage disease Incidence (PDI)

The result obtained in Table 1 below revealed that, amongst crops studied from week 1 to week 8, there is no significant difference between pepper and tomato in disease incidence. However, at week 9 and 10 tomato recorded the highest disease incidence while pepper recorded the lowest disease incidence. Probably due to their genetic architecture, temperature and varying varieties of the crop types.

However, amongst locations, Dogon Jeji and Dako-dako recorded the highest disease incidence while Digiza recorded the lowest disease incidence. This could be due to inadequate spacing pattern on their farming system which may increases the transmission and colonization of pathogen to the neighboring plants. It is clearly known that environmental condition has trigger a simultaneous effect on the disease incidence of many crops. This result was in agreement with the findings of Syed *et al.* (2014) which reported that, the maximum disease incidence due to early blight was 56.28% in F5 and minimum was 19.05% in seven days' interval. However, another finding by Sethumadhava *et al.* (2016) also reported that, the lowest percentage of disease incidence with 59.7% was found at seedling stage, whereas, the highest percentage of disease incidence at fruiting stage.

Table 1: Percentage disease incidence on major fungal pathogens of tomato and pepper in Jama'are Irrigation sites

TREATMENT	**INCIDENCE OF FUNGAL DISEASES (%) IN WEEKS**									
CROP TYPES	1	2	3	4	5	6	7	8	9	10
Tomato	21.4 [a]	26.2 [a]	33.0 [a]	36.9 [a]	44.6 [a]	50.8 [a]	54.6 [a]	59.2 [a]	59.4 [a]	59.8 [a]
Pepper	24.6 [a]	28.0 [a]	32.6 [a]	37.9 [a]	42.1 [a]	49.0 [a]	50.8 [a]	55.2 [a]	55.2 [b]	54.8 [b]
LSD	**3.80**	**4.02**	**5.17**	**6.49**	**3.38**	**4.94**	**4.15**	**4.73**	**3.29**	**4.09**
LOCATIONS										
DogonJeji	20.0 [a]	24.0 [a]	28.5 [a]	31.6 [b]	36.5 [b]	43.0 [b]	44.8 [b]	51.5 [a]	51.0 [a]	50.5 [a]
Dako-Dako	22.8 [a]	27.8 [a]	33.0 [a]	40.2 [a]	45.8 [a]	50.3 [a]	52.0 [a]	54.3 [a]	52.0 [a]	50.8 [a]
Gilar	16.8 [b]	22.3 [b]	27.3 [a]	32.0 [b]	35.8 [b]	40.5 [b]	42.5 [b]	47.5 [b]	48.8 [a]	46.0 [b]
Gongo	12.8 [b]	14.8 [c]	20.5 [b]	25.6 [b]	29.5 [c]	33.5 [c]	36.5 [c]	38.8 [c]	39.0 [b]	39.8 [c]
Digiza	10.3 [c]	12.3 [c]	16.0 [b]	18.9 [c]	22.4 [d]	27.3 [d]	27.8 [d]	30.8 [d]	32.8 [c]	34.0 [d]
LSD	**4.25**	**4.50**	**5.78**	**7.24**	**3.78**	**5.52**	**4.64**	**5.29**	**3.67**	**4.57**
INTERACTION										
Crop types*Locations	NS	NS	NS	NS	*	NS	*	*	*	*

Values within columns in the table above followed by the same letter are not significantly different at P ≤ 0.05 according to LSD.

Table 2: Percentage disease severity on major fungal pathogens of tomato and pepper in Jama'areBauchi State

TREATMENT	**SEVERITY OF FUNGAL DISEASES (%) IN WEEKS**									
CROP TYPES	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Tomato	42.1 [a]	44.2 [a]	47.6 [a]	54.5 [a]	59.8 [a]	63.7 [a]	67.4 [a]	72.1 [a]	72.3 [a]	73.9 [a]
Pepper	42.3 [a]	46.6 [a]	53.4 [a]	57.9 [a]	62.2 [a]	64.3 [a]	65.8 [a]	69.0 [a]	72.8 [a]	73.3 [a]
LSD	**13.33**	**10.89**	**11.98**	**10.31**	**10.74**	**8.91**	**8.84**	**8.17**	**6.99**	**6.53**
LOCATION										
DogonJeji	37.2 [a]	39.9 [a]	43.2 [a]	48.7 [a]	53.5 [a]	54.2 [a]	56.2 [a]	62.1 [a]	60.5 [a]	59.2 [a]
Dako-Dako	39.6 [a]	41.5 [a]	48.1 [a]	52.4 [a]	55.7 [a]	56.7 [a]	54.1 [a]	57.6 [a]	57.6 [a]	50.3 [b]
Gilar	20.0 [b]	23.0 [b]	25.2 [b]	30.7 [b]	35.4 [b]	40.0[b]	44.4[b]	47.5 [b]	49.2 [b]	48.5[b]
Gongo	23.2 [b]	30.1 [a]	34.2 [b]	39.5 [b]	45.2 [a]	47.4 [a]	51.8 [a]	54.7 [a]	56.3 [a]	57.7 [a]
Digiza	21.5 [b]	23.4 [b]	27.3 [b]	29.9 [b]	34.5 [b]	37.5[b]	39.8[b]	42.5 [b]	44.9 [b]	46.4 [b]
LSD	**14.90**	**12.17**	**13.39**	**11.53**	**12.01**	**9.96**	**9.89**	**9.14**	**7.82**	**7.30**
INTERACTIONS										
Crop types*Locations	NS	NS	NS	NS	NS	NS	NS	NS	*	*

Values within columns in the table above followed by the same letter are not significantly different at P ≤ 0.05 according to LSD

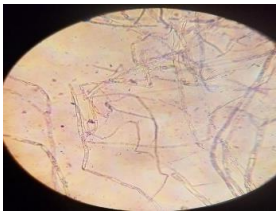
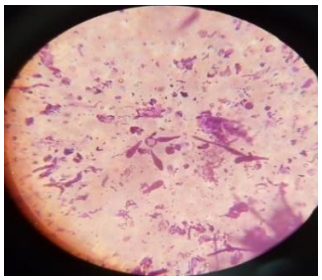
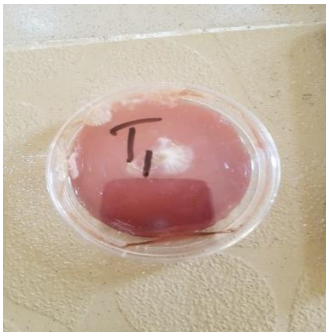
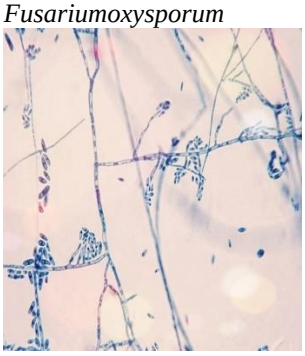
Percentage disease severity

The result in Table 2 below revealed that, amongst crop types from week 1 to week 10, there is no significant difference between tomato and pepper in disease Severity. However, amongst the locations, the maximum disease severity (39.6%, 37.2% and 23.2%) was recorded at Dogon Jeji, Dako-dako and Gongo respectively while Gilar and Digiza recorded the minimum severity (21.5% and 20.0%). The reason for

this result could be due tomato and pepper crops observed in the study area were disrupts by leaves and stem infections such as *Rhizoctonia*, *Fusarium* wilt and *septorial* leaf spot which affect the photosynthetic ability of tomatoes and pepper leaves, these will decrease the rate of carbon dioxide assimilation, also by decreasing the leaf area. However, the effect of enzymes induced by the pathogens could be the reason to increase disease severity on the crops types.

This result was in accordance with the findings of Temesgen and Sefawdin (2020) which stated that, eighteen pepper farming fields were selected for disease assessment percentage severity varies among the selected farming fields. In addition, Sethumadhava *et al.*, (2016) revealed that, the minimum disease severity was found in Awrari.

Table 3: Fungal pathogens associated with tomato (*Solanumlycopersicum*L.)

Infected portion	Colony color	Microscopic morphology	Identified pathogen	Reference
leave and fruit		mycelia were thread like, the branches of hyphae were tapered and perpendicular	*Rhizoctoniasolani* 	Sumalatha*etal.*, (2018)Amjad and Abdul Rauf (2018)
Stem and leaf		the hyphae wereseptate with branched hyaline and conidiospores having a bunch of round conidia at their tips. Conidia arose in cluster and conidiophores appeared as tree-like form.	*Botrytis cineria* 	Ahmed*etal.*,(2014) and Nawab*etal.*, (2016)
Fruit		the conidia appeared worm like with 1-4 septum, Conidiophores were singly or in group	*Alternariasolani* 	Najibullah*etal.*,(2016)
Stem and root		macroconidia were sickle shaped hyaline and multicelled with 2-3 septum, microconidia wereaseptate or singly septate.	*Fusariumoxysporum* 	Ashwathi*et al.*, (2017)Nirmaladevi and Srinivas (2012)

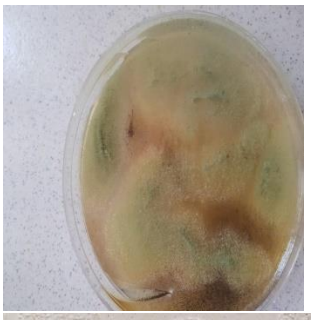
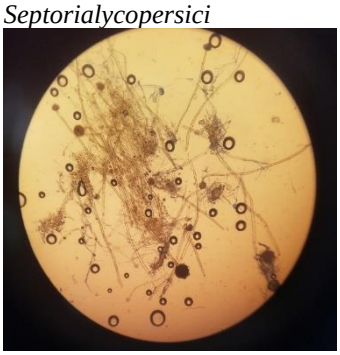
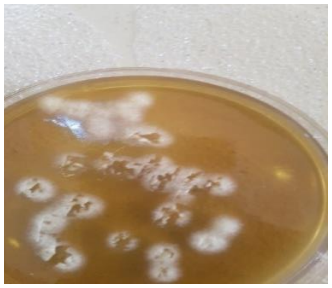
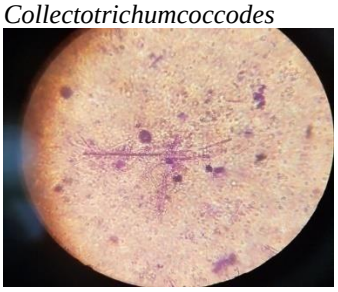
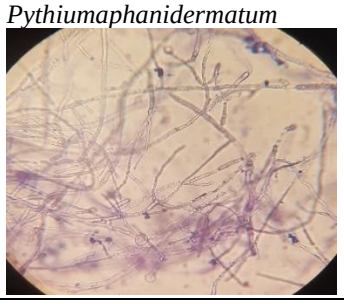
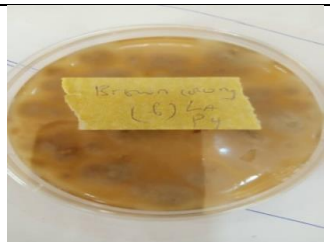
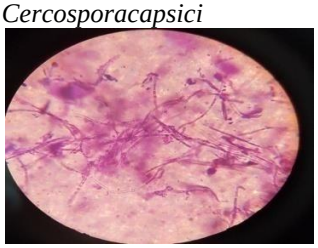
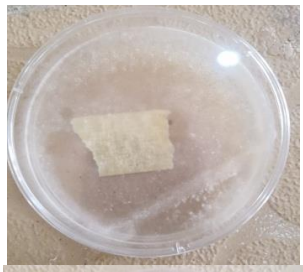
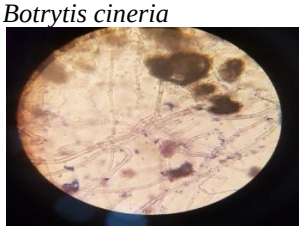
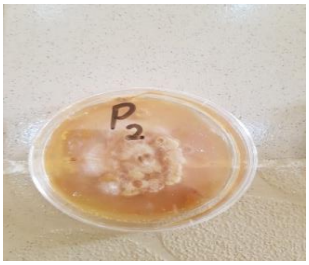
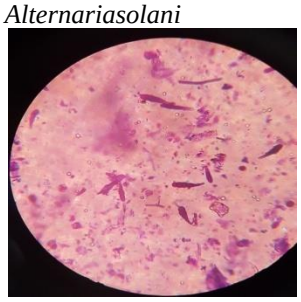
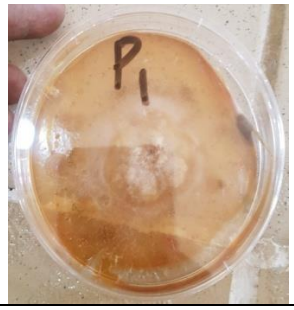
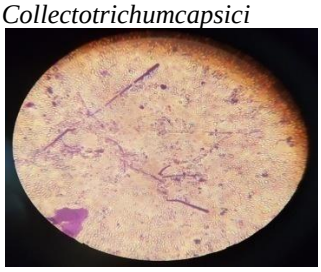
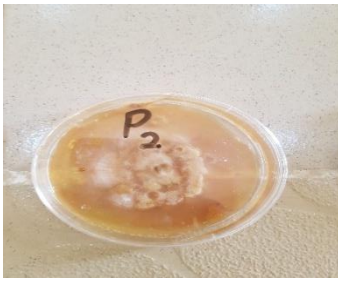
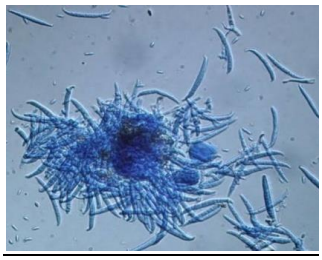
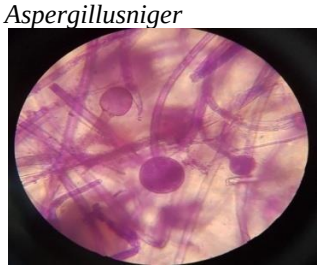
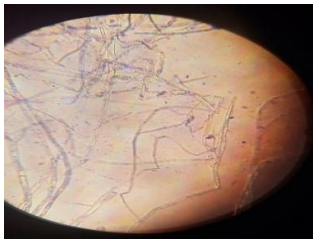
Fruit and leaf		Hyphae were filamentous, hyaline and conidial head were dark green. Conidiophore were erect and simple, Conidia were one celled spherical in shape.	*Aspergillusflavus* 	Liamngee*et al*., (2018)
Leaf		the fungus was thread like filamentous hyphae with 1-2 septum and tapered both end.	*Septorialycopersici* 	Bal*etal*., (2010)
Fruit		sporangia were hyaline and lemon shape. Sporangiophore branched with swellings at the points where sporangia attached.	*Collectotrichumcoccodes* 	Svetlana*etal*., (2010) Manoj *etal*., (2020)
leaf and petiole		Fungusappearedaseptatehyphae and dichotomously branched, hyphae had terminal or swollen structures	*Pythiumaphanidermatum* 	Motoaki *et al*., (2013)

Table 4: Fungal pathogens associated with pepper (*capsicum annum*)

Infected portion	Colony color	Microscopic morphology	Identified pathogen	Reference

Leaf		Conidiophores were short and dark, conidia were elongated with 3-5 septa	*Cercosporacapsici* 	Islam *etal.,* (2015)
Leaf		Conidia was in pseudo chain and cylindrical in shape. Conidiophores were hyaline with 1 to 4 septum	*Leveillulataurica* 	SabeenaandAshtaputre, (2019)
Leaf and stem		Hyphae were septate with hyaline conidiophores having a bunch of round conidia at their tips,Conidiophores were erect.	*Botrytis cineria* 	Faraah*et al.,* (2018)
Fruit and leaf		Funguswere elongated chain and conidiospores were dark brown. Conidia had a smooth surface with shorter beaks and contained 1-4 septum.	*Alternariasolani* 	Devappa and Thejakumar, (2016)
Fruit		Conidia was fusiform with pointed ends, while the conidia were aseptate	*Collectotrichumcapsici* 	Monaj*etal.,* (2020)

Stem		macroconidia were straight with sickled shape with 3-4 septa, and microconidia were hyaline, round to oval in shape and had about 0-1 septa.	*Fusariumsolani* 	Raghu *etal.*, (2019)
Fruit		mycelium was hyaline filamentous, conidial head were one- celled spherical and black in color, conidiophores were long and aseptate.	*Aspergillusniger* 	Hina*et al.*, (2013)
Leaf		thread like mycelia with no of spores or conidia and septum was present in the branched of hyphae near the originating point.	*Rhizoctoniasolani* 	Desvani*etal.*, (2018)

The pathogenicity result on fungal pathogens associated with tomato (Table 5) below revealed that, about six fungal pathogens had the ability to cause infection on healthy tomato which showed considerable differences among pathogens in terms of their severity, this could be due to the fact that, the pathogens invade the tissues which degrade the pectin and lignin as one of the most important structural barrier affecting plants resistance, pathogens are able to utilize the nutrients of the plants as a substrate for growth and development. However, *alternaria solani* and *Septoria lycopersici* appeared no different from control, this could be due symptoms may not be apparent at seedling stage or the inoculants are dead. This result was in conformity with the finds of Kator *et al.* (2018) who stated that, all the fungal isolates identified had the ability to cause infection on healthy tomato fruits at various percentages of severity.

Table 5: Pathogenicity test of pathogens associated with tomato and pepper

	Tomato		
Pathogens	**Inoculated portion**	**Susceptibility**	**Severity scale**
Rhizoctoniasolani	Leaves	+	2
Botrytis cineria	Leaves	+	1
Alternariasolani	Leaves	-	0
Fusariumoxysporum	Stem	+	4
Aspergillusflavus	Fruits	+	3
Septorialycopersici	Leaves	-	0
Collectotrichumcoccodes	Fruits	+	1
Pythiumaphanidermatum	Stem	+	4
	Pepper		
Cercosporacapsici	Leaves	+	2
Leveillulataurica	Leaves	-	0
Botrytis cineria	Leaves	+	3
Alternariasolani	Fruits	+	4
Collectotrichumcapsici	Stem	+	3
Aspergillusniger	Fruit	+	2
Rhizoctoniasolani	Leaves	+	2

+ symbol indicates that at least one of the replicates per fungal pathogen is severely declined compared with control while – indicates that none of the replicate died or appeared different from control.

Pathogenicity test of the fungal pathogens associated with tomato and pepper (Table 5) revealed that, about seven (6) different fungal pathogens (i.e *Cercospora capsici, Botrytis cineria, Alternaria solani, Collectotrichum capsici, Aspergillus niger, Rhizoctonia solani*) were pathogenic to pepper showing a typical symptom on the foliage and stem. This could be due to the fact, the pathogens have a wide range of host which infect the stems, leaves and fruits and secretes many cell wall degrading enzymes in the host tissues and the cells collapse, while *Leveillulataurica* appeared no different from control,this could be due symptoms may not be apparent at seedling stage or the inoculants are dead. This result was in agreement with findings of Than *et al.,* (2008) which revealed that, Pathogenicity tests with the three *Colletotrichum species* isolated from infected chilli fruits showed that all the isolates were pathogenic on the susceptible *Capsicum annuum* cultivar. This result proved that these three species of *Colletotrichum* were casual agents of anthracnose infection on chilli.

CONCLUSION AND RECOMMENDATIONS

The present research reported that, there were a total of 16 different fungal pathogens affecting tomato and pepper production in Jama'are irrigation sites whereby tomato cultivated in Dako-dako and Dogon-Jeji appeared to be more susceptible to fungal pathogens which cause serious reduction in the productivity. However, pathogenicity test revealed that thirteen (13) fungal pathogens were highly pathogenic to both tomato and pepper studied while three (3) isolated pathogens were non-pathogenic.

The current study indicated that fungal diseases exist at each growing stage of crops assessed and the occurrence across Dako-dako and Dogon-Jeji is highly severe. Therefore, an integrated approach involving massive farmer's education on the importance of crop rotation, right usage of pesticides, fertilizers and use of resistant varieties, is imperative for sustainable production, enhanced yield and improved productivity in the study area. This will alleviate annual yield loses and boost financial returns.

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