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Screening Pepper (*Capsicum annuum* L.) Cultivars for Resistance Against Fusarium Wilt Disease

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ABSTRACT

Pepper (*Capsicum* spp.) cultivation in Nigeria is significantly impacted by fungal diseases, particularly Fusarium wilt. This study aimed to screen pepper cultivars for resistance to *Fusarium* wilt in Yola, Adamawa State, Nigeria from September 2023 to June 2024. a field survey, laboratory investigations, and screen house trials were conducted.Results from the survey revealed varying levels of incidence and severity across eight study sites, with Bugare showing the highest incidence (41.3%) and severity (25.6 %), and Geriyo the lowest (16.7% and 12.5% respectively). *Fusarium solani* and *Fusarium oxysporum* were isolated from infected pepper samples, and pathogenicity test confirmed their ability to induced disease symptoms. Among the isolates, *Fusarium oxysporum* was the most frequently isolated fungus, occurring in 91.7 % of the diseased samples collected. Significant differences (p=0.05) were observed among the ten pepper cultivars screened for resistance. CultivarTDKS washighly susceptible (100% incidence, and56.7% severity), while cultivars TDZR, ADGE, and SDGJ demonstrated moderate to high resistance, with Disease Severity Index (DSI) values below 20% for *F. oxysporum* and between 20-39% for *F. solani*. The study concludes that Fusarium wilt poses a serious threat to pepper cultivation in Yola, with TDZR being the most resistant cultivar. This cultivar could serve as a valuable genetic resource in breeding program to develop Fusarium wilt-resistant varieties.

Keywords: Pepper Cultivars, Resistance, Fusarium wilts, *Fusarium solani*, *Fusarium oxysporium*

INTRODUCTION

Pepper, scientifically known as *Capsicum* spp., is a vegetable crop belonging to the Solanaceae family and Capsicum genus. Pepper is believed to have originated from tropical America (Aliyu *et al*., 2012). In Nigeria, pepper consumption was once reported to be forty percent of the total vegetables consumed per day especially for poor people (Rodrigues *et al*., 2012). Farmers worldwide cultivate pepper for both food and economic purposes, with its demand significantly rising in recent years (International Pepper Conference (IPC) Proceedings, 2012). Nigeria and Ghana are the largest pepper producers in West Africa, ranking eighth and thirteenth globally (FAOSTAT, 2020). Despite having fertile soils, favorable weather, and available land, Nigeria's reported yield of 8.4 t/ha is lower than the estimated yields of 15 t/ha in Western Europe. Production constraints such as diseases, pests, and poor weed management were initially blamed for the low yields (Jaliya and Sani, 2006; Zakari *et al*., 2016).

Peppers in Nigeria and other sub-saharan Africa face various diseases caused by fungal pathogens, posing threats throughout their

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growth stages, transportation, marketing, and storage (Olawale *et al*., 2015; Asare-Bediako *et al*., 2015; Zakari *et al,* 2016). Fungi, especially *Fusarium* species, contribute to for the low yields in pepper production in Nigeria (Jaliya and Sani, 2006; Zakari *et al*., 2016; Zakari *et al*., 2024). In view of the above problems and the need to understand the dynamics of fusarium wilts disease in pepper cultivation within the region, this research work was designed to screen pepper cultivars available in Yola against Fusarium disease.

MATERIALS AND METHODS

The Study Area

This research was carried out in Yola, Adamawa State (Figure 1), Nigeria, spanning from September 2023 to October 2024. The study areas were situated within the Guinea savannah ecological zones, characterized by distinct rainy season and dry seasons. The geographical coordinates range from latitudes 8º47' to 9°19'N and longitudes 11º09' to 12°30'E. According to the data obtained from Upper Benue River Basin Development Authority (UBRBDA) (2020), the wet season typically extends from late April to October, while the dry season spans November to March. Yola experiences an annual mean rainfall of about 1,200 mm, a mean temperature of approximately 29ºC, and varying relative humidity, ranging from 60–70% in the wet season to 35–45% in the dry season. The soil composition in these areas is predominantly sandy and loamy.

Experimental Design

The research design comprised a field survey and experimental studies following the method described by Asare-Bediako *et al*. (2015) during the 2023 production seasons. The survey used the field disease guide (2014) to assess the incidence and severity of Fusarium wilts disease in pepper across pepper-growing villages in Yola, including Bugare, Geriyo, Malkohi, Namtari, Ngorore, Wafango, Wuro Ishaku, Wulum. The experimental design utalized a completely randomized design (CRD) with 8 local pepper cultivars established during the pilot study. The experiment was conducted in three replicates.

Survey on the Assessment of Fusarium Wilts Disease of Pepper in Yola

A total of twenty-four survey farms were randomly selected, with three farms from each site mentioned above. Disease assessment involved visually inspecting plants for symptoms of Fusarium wilts disease, as described in the Field Guide to Pepper Diseases and Disorders (2014). Observations were recorded on field survey record sheets, and photographs and videos documented the disease materials. Disease incidence (DI) was calculated as the percentage of infected plants per farm relative to the total number of observed plants. The disease severity index (DSI) for each disease was computed using the formula DSI = Σ (P x Q)/ (M x N)] x 100, where P is the severity score, Q is the number of infected plants with the same score, M is the total number of plants observed, and N is the maximum rating scale number.

Isolation of fungal pathogens

Diseased pepper fruits, leaves, roots and stems of the three cultivars (Tattase, Atarugu and Shambo) collected from the field were surface sterilized by dipping them in 90 % ethyl alcohol for one minute and then rinsed in three changes of sterile distilled water. Small pieces of infected tissue (2-3mm in length) were cut at a point between diseased and healthy portion with the help of disinfected blade after surface sterilizing the sample with alcohol. These bits were surface sterilized in 0.1 percent mercuric chloride solution ($HgCl_2$) for 30 seconds followed by three washings with sterilized distilled water

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in Petri dishes under aseptic conditions. These bits were then dried by placing on sterilized blotting paper. Three bits were transferred aseptically to the Petri dishes containing sterile potato dextrose agar (PDA) medium.

Soil samples were also collected, air-dried, grinded lightly and mixed thoroughly. Serial dilution ratios 1:10, 1:100 and 1:1000 of soil sample to sterile distilled water were made. One milligram of the solution was pipetted carefully onto the edge of the medium to one side of the plate. Inoculated Petri plates were incubated at 25 ± 2℃ for seven days and examined.

The frequency of isolations was determined using the percentage frequency of isolation as follows:

$$\%\text{Frequency} = \frac{\text{Number of times a fungus encountered}}{\text{Total fungal isolated}} \text{x } 100$$

Maintenance and preservation of pure culture

The fungi isolates were further maintaned by single hyphal tip method. The fungi were grown by inoculating in the centre of a plain agar plates. The fungi were spread out with their hyphal stands in search of nutrients. These hyphal strands were observed under low power of the microscope in the inverted petri-plate and the isolated hyphal tips marked with marker. These tips were carefully transferred to potato dextrose agar slants and maintained at room temperature 25 ± 2℃ for 10 days. The pure cultures were then preserved in the refregirator at 4 ° C in the laboratory.

Preparation of fungal suspensions

Fungal suspensions were prepared following the procedures reported by Burgess (2008). Seven day old cultures of the fungal isolates were flooded with 25 ml of sterile distilled water. With the aid of a carmel hair brush, the spores were carefully brushed off the sporophores and decanted into a sterile petri dish. Fifteen millilitre of sterile water was added and filtered through 0.2×0.2 mm nylon mesh to get rid of mycelial fragments. This filtrate containing the spores was adjusted to a concentration of 1×10^6 spores per ml through the addition of some quantity of sterile distilled water.

Acquisition of Pepper Genotypes

Eight pepper cultivars Atarugu Dan Kasa, Atarugu Dan Ganjuwa, Atarugu Dan Gembu, Atarugu Dan Gombe, Tattatse Dan Kasa, Tattatse Dan Ganjuwa, Shambo Dan Kasa and Shambo Dan Ganjuwa of locally collected accessions from Yola, Nigeria and Tattase Dan Zaria, Tattatse Dan Borno collected from National Horticultural Research Institute (NIHORT) Kano substation were evaluated for their reaction to Fusarium wilt.

Screening of Pepper Cultivars for Resistance Under Laboratory Conditions

Ten *Capsicum* cultivars collected (listed above) were screened against *Fusarium oxysporium* and *Fusarium solani* on detached pepper fruits and leaves in the laboratory. Partially riped mature fruits and leaves were carefully detached from plants and washed with sterile distilled water (SDW) and then wiped with cotton wool soaked in ethanol to reduce microbes on the surface. Artificial inoculation was performed on samples by injecting 2 ml spore suspensions of the fungal pathogens and fruits were covered with polythene to create humid condition for disease development. Polythene was removed after 24 hours and samples were incubated for 3 days at room temperature to observe disease lesions development. The diameter of the lesions that developed on and around the

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puncture wound at 3 days after inoculation was recorded. Disease incidence was evaluated using Yoon (2003) method. The rating system used was based on incidence of diseases as follow:

$$DI = \frac{n}{N} X\ 100\%$$

Where;

DI = diseases incidence; n = infected fruits with lesion diameter; N = total of fruits inoculated. The grading was done on the basis of disease incidence (%) as:

0. 0 = Immune (I)

1. $0 \leq X < 10$ highly resistant (HR)

2. $10 \leq X < 20$ Resistant (R)

3. $20 \leq X < 40$ moderately resistant (MR)

4. $40 \leq X < 70$ Susceptible (S)

5. $X \geq 70$ highly susceptible (HS)

A cultivar was selected for next line of screening only when graded 1-3 in all the pathogens tested. The most susceptible cultivar was also selected to check its susceptibility in the screen house control.

Screening of Pepper Cultivars for Resistance Under Screen House Conditions

Twelve plants were used for each cultivar (i.e. a total of 72 plants were used for the whole experiment). For inoculation, the *Fusarium oxysporium* and *Fusarium solani* isolates were used. Ten-mls of spore suspension prepared as described above was poured in three holes made around roots of 45 days old pepper seedlings in each pot inoculated with the pathogens. Seedlings were immediately capped with transparent polythene bag to create humid condition for disease development. Seedlings were uncapped and remained in the screen house to evaluate the reaction of accessions with the control seedlings treated only with distilled water.

The plants were monitored every two weeks for disease symptoms of Fusarium wilts. Then the disease was evaluated based on different criteria. Disease severity rating scale was rated based on a 5-point scale as per Asian Vegetable Research and Development Centre (AVRDC) pepper disease compendium (AVRDC, 2003). Such as 0=no visible infection, vigorous, healthy=R; 1=slight leaf yellowing=MR; 2=old lower leaf yellowing and plant wilting=MS; 3=lower leaves shading and stunted plants=S; 4=all the leaves shedding and the stem collapsed and few plants death and HS; 5=Total plant death. Disease incidence (DI) was calculated as the proportion of infected plants per plot and expressed as a percentage (Singh and Singh, 2001) and grading was done on the basis of wilt incidence (%) as described above.

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) and standard error of means using Paleontological Statistics (PAST) package version 4.07 and means were separated using Fischer's Least Significant Difference (FLSD) at 5 % probability level.

RESULTS

Incidence and Severity of Fungal Diseases in Pepper Farms in Yola

Results from the survey revealed varying levels of incidences and severities of Fusarium wilt disease of pepper in various study sites in Yola (Table 1). The incidence and severity of the disease varied significantly ($p=0.05$) among the eight study sites. Bugare has the highest incidence and severity of the disease, with an average incidence of 41.3% incidence and a mean severity of 25.6 %. Ngorore followed with 34.7 % incidence and 23.4% severity. Geriyo recorded the lowest incidence and severity of Fusarium wilts, with an incidence of 16.7 % and a mean severity of 12.5%.

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Isolation and Identification of Fungal Pathogens on Pepper Plants

Two fungi were isolated from different parts of diseased pepper plants collected from various pepper fields in the eight study sites in Yola. The fungal Isolates were identified based on their characteristics, appearance on culture media, and under a light microscope as *Fusarium oxysporum* and *Fusarium solani* (Table 2 and Plates 1-4). The Frequency of isolation of fungi is presented using in Table 3. Among the isolates, *Fusarium oxysporum* was the most frequently isolated fungus. Occurring in 91.7 % of the diseased samples collected. It was present in all the four plant parts examined at both seedling and maturity stages, but was absent in the fruits of plants examined at flowering stage, appearing 11 times out of a possible 12. *Fusarium solani* had 50.0 % frequency of isolation, appearing in 6 out of 12 total isolations.

Screening of pepper cultivars for resistance against pathogens under laboratory condition

Significant differences were observed among the ten pepper cultivars screened for resistance against the two pathogenic fungi *Fusarium oxysporium* and *Fusarium solani*. The results (Table 4) revealed that cultivar TDKS had the highest disease severity with 56.7% disease severity and 100% incidence and was rated highly susceptible, ranking 5 on the 0-5 scale. Cultivars TDZR, TDBN, ADKS and SDGJ had disease severities of less than 50% and were ranked 3 on 0-5 scale, indicating moderate resistance to Fusaruim rots. The cultivar ADGE was ranked 2 on the 0-5 scale and rated resistant. The remaining three cultivars, TDGE, TDGJ and SDGJ were rated as either highly susceptible or susceptible, with disease severities ranked 5 or 4 on the 0-5 scale.

Table 1: Incidence and Severity of Fusarium Wilt Disease on Pepper Surveyed in Yola during the 2023 Rainy Season.

Producing Area	Mean disease incidence (%)	Mean disease severity (%)
BGR	41.3	25.6
GRY	16.7	12.5
MKH	20.0	13.1
NGR	34.7	23.4
NTR	22.0	16.1
WFG	22.7	14.7
WLM	24.0	15.8
WRI	20.0	16.9
Mean	25.2	17.3
p-value	0.045	0.040
LSD	14.48	Ns

BGR-Bugare, GRY-Geriyo, MKH-Malkohi, NTR-Namtari, NGR-Ngorore, WFG - Wafango, WRI-Wuro Ishaku, WLM-Wulum. Data are means of three farms (replicates).

Table 2: Characteristics of Fungi Isolated From Pepper in Jalingo and Yola during 2023 Rainy Season.

Fungal pathogen	Cultural characteristics	Morphological characteristics
Fusarium solani	Colony on PDA has abundant aerial mycelium, cream surface or purple–coloured with undersurface showing a dark violet color or colorless.	Identification was based on microconidia formed from lateral long monophialides, narrowing at the apex, unicellular, oval or kidney–shaped. Macroconidia were generally cylindrical almost the entire length, with 3–5 septate. Chlamydospores are formed singly or in pairs, globular or oval, with a smooth or wrinkled wall.
Fusarium oxysporium	Colony on PDA initially with white aerial mycelium, becoming salmon, with a tendency towards violet, and a purple back.	Microconidia produced on short monophialides as a false head, mostly unicellular, varying from oval–ellipsoid to cylindrical and from straight to curve. Macroconidia are also formed in abundance, with an attenuated apical cell and a pedicellate basal cell, generally with 3–5 septate, produced in short branched or unbranched monophialides or sporodochia. Another striking characteristic observed

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was the constant presence of chlamydospores, with a smooth wall, the most formed singly, with intercalated or terminal location.

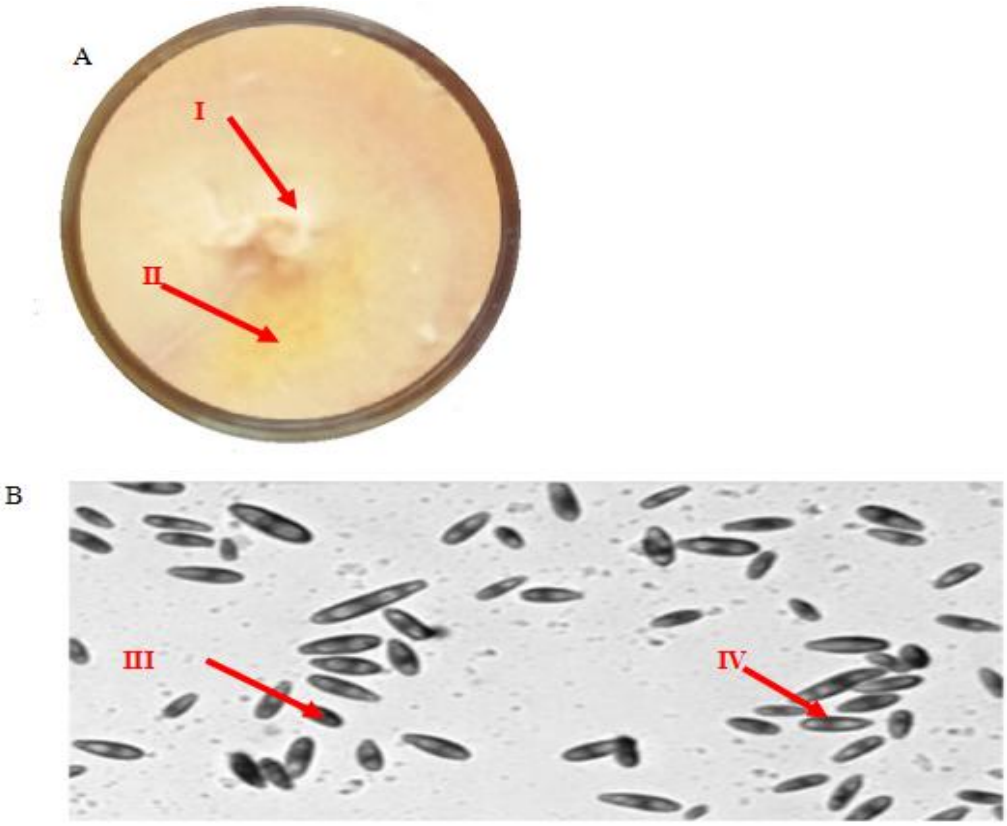

Plate I and II: Cultural and morphological characteristics of a seven-day-old *Fusarium oxysporum* (A-front plate, B- Conidia at x10 objective, I &II- Salmon old and white advancing mycelia, III&IV- micro and macro conidia).

Table 3: Percentage Frequency of Fungi Isolated from Pepper Plants at Different Stages of Growth in Yola During the 2023 Rainy Season.

Growth stage Isolates	Seedling stage				Flowering stage				Maturity stage				Occurrence		
	PR	PS	PL	RS	PR	PS	PL	RS	PR	PS	PL	PF	Total isolation	Frequency	Percentage
F. oxysporum	√	√	√	√	√	√	√	×	√	√	√	√	12	11	64.7
F. solani	√	√	×	√	√	×	×	×	√	√	×	×	12	6	35.3

Key: PR-Plant root, PS-Plant stem, PL-Plant leaf, PF-Plant fruit, RS-Root soil, √ = Ditected, × = not ditected.

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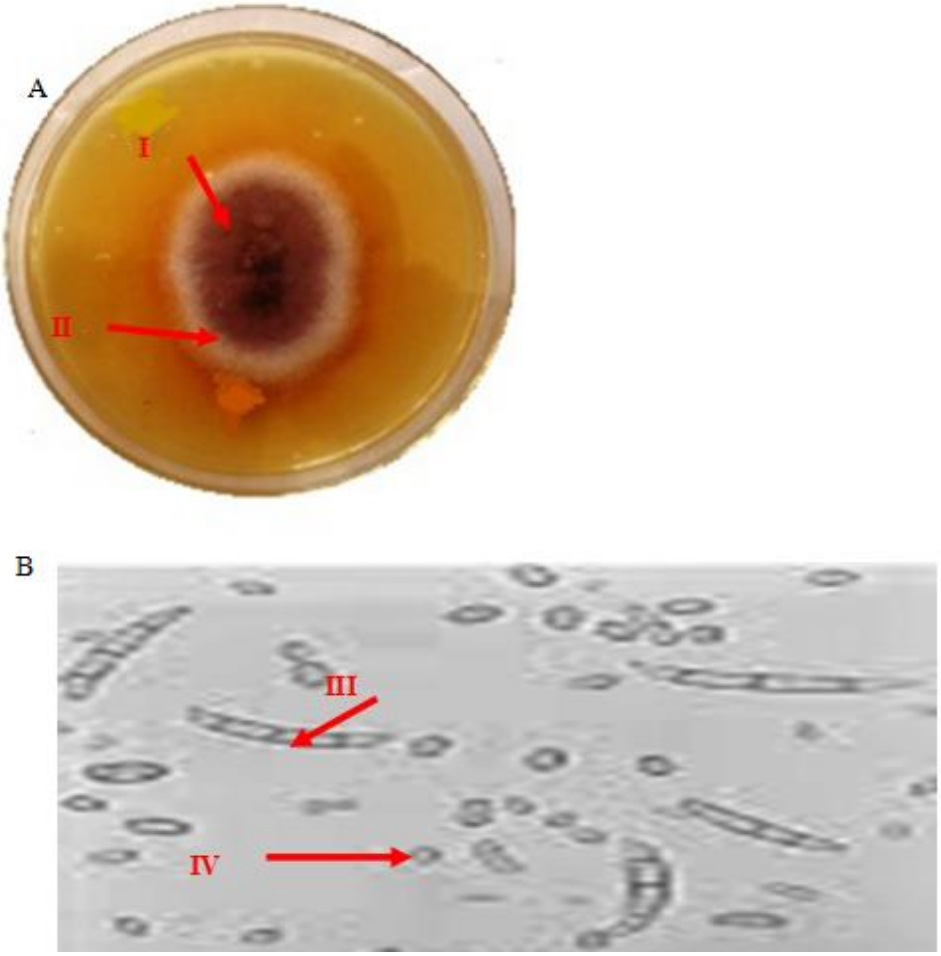

Plate III and IV: Cultural and morphological characteristics of a seven-day-old *Fusarium solani* (A-front plate, B- Conidia at x10 objective, I &II- pink old and white advancing mycelia, III&IV- micro and macro conidia).

Table 4: Incidence and Severity of Fungal Diseases on Fruits of Pepper Cultivars under *In vitro* Condition.

	***F. solani* (Fusarium rot)**				**Control (other rots)**	
Cultivar	**DI**	**DS**	**DR**	**HR**	**DI**	**DS**
TDGE	80.0	26.7	5	HS	10.0	5.9
TDKS	100.0	56.7	5	HS	30.0	5.3
TDZR	30.0	20.0	3	MR	20.0	6.6
TDBN	30.0	35.6	3	MR	40.0	4.4
TDGJ	50.0	30.0	3	S	20.0	6.3
ADKS	40.0	32.8	3	MR	10.0	4.2
ADGE	20.0	26.7	2	R	20.0	4.4
ADGB	60.0	42.6	4	S	20.0	3.2
SDGJ	40.0	15.9	3	MR	0.00	0.0
SDKS	80.0	42.8	5	HS	10.0	3.8
Mean	53	33.0			18	4.4
p-value	<.001	<.001				
LSD	25.79*	11.72 **				

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Key: TDGE-Tattase Dan Gombe, TDZR-Tattase Dan Zaria, SDGJ-Shambo Dan Ganjuwa, SDKS- Shambo Dan Kasa, TDKS-Tattase Dan Kasa, TDGJ- Tattase Dan Ganjuwa, TDBN--Tattase Dan Borno, ADKS-Attarugu Dan Kasa, ADGE-Attarugu Dan Gombe, ADGB-Attarugu Dan Gembu DI= Disease incidence, DS= Disease severity index, DR= Disease rating, HR= Host response, S= Subceptible, HS= Highly subceptible, R= Resistant, MR= Moderately resistant, MS= Mederately subceptible ***=** LSD for DI, **=** LSD value for DSI.

None of the cultivars evaluated were completely immune to the pathogens tested on detached tissues. Five cultivars with at least a moderately resistant trait were selected for screen house trials, while the susceptible cultivar TDKS was chosen as susceptibility check.

Screening of pepper cultivars for resistance against pathogens under screen house condition

The study was conducted in screen house to evaluate pepper cultivars that passed the preliminary screening against pathogens under *in vivo* conditions. Significant differences were observed among cultivars in terms of wilt disease incidence and severity indices (Table 5). Among the 6 pepper cultivars screened, only three -TDZR, ADGE and SDGJ were found to be resistant to Fusarium wilt pathogen *F. oxysporium* (with disease severity index (DSI)<20) and moderately resistant to *F. solani* (with a DSI of 20-39). Cultivar TDBN was moderately resistant to both two pathogens, while cultivar ADKS was moderately resistant to *Fusarium solani* (with DSI >50%). As in the *in vitro* trials, susceptibility checks TDKS remained susceptible to both pathogens under the *in vivo* conditions.

Table 5: Incidence and Severity of Fungal Diseases on Pepper Cultivars in the Screen house.

	F. solani **(Vascular stem rot)**				***F. oxysporum*** **(Vascular wilt)**				**Distilled water**	
Cultivar	**DI**	**DSI**	**DS**	**HR**	**DI**	**DSI**	**DS**	**HR**	**DI**	**DS**
TDKS	100.0	56.7	3	MS	55.6	42.2	3	MS	16.7	3.3
TDZR	33.3	20.0	2	MR	33.3	13.3	1	R	16.7	3.3
TDBN	44.4	15.6	2	MR	66.7	26.7	2	MR	0.0	0.0
ADKS	55.6	40.0	3	MS	50.0	23.3	2	MR	11.1	2.2
ADGE	83.3	26.7	2	MR	11.1	8.9	1	R	22.2	4.4
SDGJ	44.4	15.6	2	MR	16.7	6.7	1	R	0.0	0.0
Mean	60.2	29.1			38.9	20.2			11.1	2.2
p-value	<.001	<.001								
LSD	13.93*	24.95**								

Key: TDBN-Tattase Dan Borno, TDZR-Tattase Dan Zaria, ADGE-Atarugu Dan Gombe, TDGB-Tattase Dan Gembu, SDGJ-Shambo Dan Ganjuwa, TDKS-Tattase Dan Kasa, DI= Disease incidence, DSI= Disease severity index, HR= Host response, R= Resistant, MR= Moderately resistant, MS= Mederately subceptible ***=** LSD for DI, ** = LSD value for DSI.

DISCUSSION

There were high prevalence and severity of Fusarium wilt disease in Yola and *Fusarium oxysporium* and *Fusarium solani* were the fungi isolated from pepper samples collected across the pepper cultivation fields in the area. The above diseases together with phytophthora leaf blight and cercospora leafspot diseases have also been identified on pepper fields in other parts of Africa, in Nigeria (Olawale *et al*., 2015; Zakari *et al*., 2015; 2016; 2024), in Uganda (Nsabiyera *et al.*, 2012), and in Ghana (Asare-Bediako *et al*., 2015) confirming the prevalence of these diseases in pepper fields in sub-Saharan

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Africa. This finding could partly account for the low yield of pepper recorded in Yola, and hence the disease is a major threat to pepper production in this area. Fungal diseases have been reported to cause more than 50 % losses of marketable pepper fruits in Nigeria (Jaliya and Sani, 2006; Zakari *et al*., 2015; 2024).

Among the isolates, *Fusarium oxysporum* were the most frequent fungi isolated from diseased samples collected in the study sites appearing in all the parts examined. In another study aimed at assessing the occurrence of fungi responsible for postharvest losses in the most common varieties of *Capsicum* peppers collected from retail markets in Nigeria and Ghana found *Aspergillus* spp., *Fusarium* spp., and *Colletotrichum* spp. predominant fungal pathogens (Akinyemi, 2000; Lema *et al*., 2018). The consistent appearance of *Fusarium* species in above studies explains why *Fusarium* species were the fungus found most frequent in this study. It could also be due to the fact that *Fusarium* species have wide host range including tomato and other solanaceous crops, sweet potato, legumes, cucurbits and banana which are the most susceptible plants (Lema *et al*., 2018). These plants were found mixed cropped with papper in the study area.

Results from laboratory screening of pepper cultivars for resistance against Fusarium wilt pathogens revealed that local cultivars were susceptible to Fusarium wilt disease. The result is in line with finding by Temiyakul *et al.* (2012) who reported that no commercial resistant varieties of *C. annuum* have been developed, due to the lack of resistance in the *C. annuum* gene pool. The potential for disease resistance as a control measure for pepper has been suggested in studies based on observations carried out either under field conditions or with artificial inoculation techniques (Hasyim *et al*., 2014). AVRDC (2003) reported that use of host plant resistance to control plant diseases was the most important method of the disease management strategies. It further reported that genotypes resistant to different fungi species and pathotypes (virulent isolates) within species have been used to study inheritance of resistance in many crops. Host resistance in these species has been shown to be differentially expressed at different fruit maturity stages (Montri *et al*., 2009; Mongkolporn *et al*., 2010). Ko *et al*. (2005) reported on differential resistance in green and riped fruit of the same pepper variety as revealed by high expression of pepper esterase (*PepEST*) gene, while Manandhar *et al* (1995) reported that disease incidence was correlated to cuticle and exocarp thickness and varied by fruit maturity. Prasath and Ponnuswani (2008) reported that phenol and enzyme play a role is resistance against fungal infection on chili pepper.

Among the 6 pepper cultivars screened in the screen house, only three cultivars TDZR, ADGE and SDGJ were found moderately resistant to Fusarium wilt pathogens. The present study is in agreement with the reports of Joshi *et al*. (2015) which indicated great disease incidence variation within the range of 0 to 78.7% and from thirty pepper varieties screened, only two varieties were found 100 % resistant, against Fusarium wilt of pepper and Aklilu *et al*. (2018) from forty-nine varieties against vascular wilt of pepper.

CONCLUSION

It may be concluded from this study that there are high prevalence and severity of Fusarium wilt disease on pepper in the study area. *Fusarium oxysporum,* and *F. solani* were the pathogens associated with the Fusarium wilts disease in the study areas with *F. oxysporum* the most isolated among the two fungi. Tattase Dan Zaria was the most resistant pepper cultivar found in the study area. In view of the above findings, it is recommended

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that: High resistance to vascular wilt found in TDZR cultivar can be used as the source of resistance in breeding programme to develop resistant varieties for vascular wilts. *In vitro* screening of this cultivar against the tested pathogen using plant tissue culture could be conducted to ease breeding activity to develop resistance line since it is easier to handle, consume less resources and more accurate than the field trial.

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