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RESEARCH ARTICLE

BACTERIAL AND FUNGAL PATHOLOGIES OF TOMATO (*SOLANUM LYCOPERSICUM* L.): STATUS OF THE DISEASES AND CONTROL PRACTICES OF FARMERS IN TOGO

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Abstract

Tomato (Solanum lycopersicum L.) is of great socio-economic Tomato (Solanum lycopersicum L.) is of great socio-economic importance. Unfortunately, its production is limited in Togo by fungal diseases and bacterial wilt caused by *Ralstonia solanacearum* (Rs). The objectives of the study are to establish the status of major tomato diseases in Togo and control approaches used by the producers. The methodology led to a field survey allowing the evaluation of the incidence of fungal and bacterial diseases of tomato and the identification of their endogenous control methods. In the laboratory, the isolation and identification of the pathogens responsible for these diseases were carried out. The results show that the major diseases of tomato in Togo were, spots, organ decay and wilting plants. The incidence rate of fungal diseases ranges from 18.8% to 75% (fields) and from 0% to 15% (plants). The pathogens responsible are Sclerotium rolfsii (7.5%); *Sclerotinia sclerotiorum* (1.5%), *Alternaria solani* (2.5%) and *Fusarium oxysporum* (4.38%). The incidence rate of Rs wilt is 100% (field) and ranges from 10% to 43.33% (plant). The PCR test revealed that the bacterium responsible for tomato wilt in Togo is Rs belonging to phylotype I, Séquévar 17. Synthetic chemical pesticides are used to control these pathologies, which is a problem for human and animal health and environmental protection. Chemical pesticides have been identified, 60% of which are insecticides and 30% fungicides. The study revealed unproper practices in the management of pesticides in region covered by the research, leading to sanitary risks for farmers.

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Introduction:-

Tomato (*Solanum lycopersicum* L.) is of great socio-economic importance. Unfortunately, tomato fruit yield is low with 12 t/ha (DSID, 2019) compared to world yield of 25-30 t/ha (FAO STAT, 2019). The production of tomato in 2019 was 321,644 tons in Benin (Mensah et al., 2019); 2.3 million tons in Nigeria (FAO STAT, 2019); 300,000 tons in Burkina Faso (DGPV, 2019); 240,000 tons in Niger (FAOSTAT, 2019) and 11,589 tons in Togo (DSID, 2019). These low yields are due to various constraints, among which diseases are the major factors (Kunwar and Bamazi et al., 2021) resulting in yield losses of up to 60% or even 100% (Bamazi, 2017; Sikirou et al., 2001).

Many diseases affecting tomato have been described worldwide (Blancard et al., 2009), in Benin (Sikirou et al., 2017). In Togo, besides the work of Dansou-Kodjo et al. (2017) on tomato virus diseases and Ayisah et al. (2019) on tomto wilt, the inventory of tomato diseases is still limited. However, farmers are faced with vegetable diseases, especially the general wilting of the tomato plants at all stages and mainly at flowering or fruiting once.Thus, Kunwar and Bamazi et al. (2021) reported the occurrence of bacterial wilt of tomato and the pathogen *Ralstonia solanacearum* was isolated and caraterazed. Moreover, farmers practices in pesticides use has been investigated in Togo, but limitted to peri-urban area of Lome. In the frame work of determining the status of tomato diseases and developping suitable control measures countrywide, the present study aimed to survey the bacterial and fungal diseases of tomato, identify the pathogens and the control practices used by the farmers.

Materials and Methods:-

Study area and diseases survey

The study area is the Central Region of Togo in West African zone. The country is located between 6° and 11° North latitude (Gù-Konu et al., 1981) and has 95.8% land and 4.2% water (Amegadje 2007). Monthly average temperatures range from 23.1°C to 26.7°C over the year. Minimum and maximum temperatures range from 20.5°C to 23°C and 26.2°C to 33.7°C respectively. The extreme values of the relative humidity of the atmosphere are 43% in January and 88% in September (METEO-Togo, 2020). The annual rainfall ranges from 1100 mm to 1400 mm (ICAT-Centrale, 2020). The survey covered three prefectures, Sotouboua, Tchaoudjo, Tchamba and Blitta with a total of 16 localities.

Sampling of localities and fields

The tomato fields surveyed were selected based on the directory of vegetable growers and large tomato production areas available at the of the « Institut de Conseil et d'Appui Technique de la Région Centrale (ICAT-Centrale) ». The survey involved 4 prefectures, 4 localities in each prefecture and 3 fields in each locality. A total of 48 fields were visited over the 16 localities.

Disease diagnosis

In each field, the diagnostic consisted of the analysis of major symptoms observed on the plants following the methods used by Banito *et al.* (2007) and Blancard *et al.* (2009). For wilt symptoms, streaming and immunostrip tests were carried out (Banito, 2003; Paret *et al.*, 2011; Sikirou *et al.*, 2015).

Disease incidence at field level

Field-level incidence or prevalence is the ratio of the number of infected fields - where the disease is observed – over the total number of fields visited (Banito, 2003; Mwanik *et al.*, 2016; Ayisah et al., 2019; Bamazi et al., 2019). This incidence was calculated using the following formula:

$$\mathrm{IF} = \frac{\mathrm{FD}}{\mathrm{FT}} * 100$$

with IF = field-level incidence ; FD = number fields with diseased plants ; FT = total number of fields visited.

Disease incidence at plant level

In each field, three plots of 50 m^2 were delineated for disease incidence assessment at plant-level (Sikirou et al., 2017; Bamazi et al., 2019). The incidence was calculated as follows :

$$IP = \frac{PF}{PT} \times 100$$

where: IP = Plant-level incidence; PF: Number of diseased plants; PT: Total number of plants observed plants.

Media preparation for pathogen isolation

Three standard crop media types were used: Potato Dextrose Agar (PDA) for fungi isolation, Nutrient Agar (NA) for bacteria isolation. These media were prepared as described by Fox (1993), Banito (2003) and Elphinstone (2005) and Sikirou et al. (2015). For *Ralstonia solanacearum* isolation,Modified Semi-Selective Medium from South Africa (M-SMSA) was used (Denny *et al.*, 2001; Balamurugan *et al.*, 2020; Kunwar and Bamazi *et al.*, 2021)

Isolation and identification of fungal pathogens

Fungi were isolated using the method described by Fox (1993) and Walker (1998). Identification of fungal pathogens was based on macroscopic characteristics (Guiraud, 1998) and microscopic characteristics (Kirk et al., 2014; Botton et al., 1990).

Isolation and identification of bacterial pathogens

For bacterial symptom, samples were washed with tap water, disinfected in a 1% bleach solution for 1 min, and then rinsed properly with sterilized distilled water (Kini, 2018 and Balamurugan et al., 2020). For each sample, fragments of leaves showing the young symptoms of necrotic spots were cut into small pieces and put into a few drops of sterilized distilled water in a petri dish and incubated for at least five minutes. A loopful of macerate solution was stricked on NA in petri dishes and incubated for 48-72 h at 28°C (Banito 2003; Banito et al., 2012; Sikirou et al. 2017 and Balamurugan et al. 2020).

For wilted plants suspected as bacterial wilt, two preliminary tests - streaming and immunostrip - were performed prior isolation.The streaming test or water glass test, the stem of wilted plant was rinsed with tap water, cut above the collar and plunged vertically with the end cut into a test tube/glass containing sterile distilled water. After 2-5 minutes, bacterial production flowed when positive (Yabuuchi et al., 1995, Junaid et al., 2019). These milky-white flows are characteristic of the presence of *R. solanacearum* in the vascular system of the plant (Priou et al., 1999).

The immunostrip test was performed using Agdia's *R. solanacearum* specific immunostrip kit ISK33900/0025 following the method described by Denny *et al.* (2001) and used byParet *et al.* (2011), Balamurugan *et al.* (2020) and Kunwar and Bamazi *et al.* (2021). For this, 2 to 3 cm long fragment of the wilted tomato plant was mcerated into the buffer solution of the serological kit. Then, the extract was well homogenized with the buffer solution and the *R. solanacearum* specific drive stick from the kit was introduced into the homogenate. The result displayed after 2 to 3 minutes on the player by the appearance of a single or two bands depending on whether the result is negative or positive (Paret et al., 2008, 2011).

For the positive samples, the bacteria were isolated, purified and processed for PCR in a separate investigations carried out by Kunwar and Bamazi *et al.* (2021). Thus the corresponding results are not included in the present paper.Pathogenicity testing: Four strains of *R. solanacearum* from the 4 prefectures were used for the pathogenicity test as described by Sikirou et al. (2017): T18 from CECO in the prefecture of Sotouboua, T35 from Sabaringadè in the prefecture of Tchaoudjo, T40 from Albi1 in the prefecture of Tchamba and T49 from Kélébo in the prefecture of Blitta. A bcterial suspension of 10^7 CFU/ml was prepared from the 72-hours pure cultures of each of the strains. Twenty-one Tomato plants of 21-days old of the susceptible variety in plastic pots were inoculated with 50 ml of the bacterial suspension per plant under greenhouse conditions at the experiment station of ESA/UL. Five plants were inoculated for each strain. The control plants received distilled water. The assessment consisted of daily observation of the plants until the onset of wilting symptoms.

Pesticides assessment from producer
During the survey, the phytosanitary products used for diseases control were assessed by interviewing farmers. The updated list of the phytosanitary products approved in Togo (DPV, 2021) allowed discriminating the products used in pest control by farmers.

Statistical analysis
The collected data was formated into Excel software, and analysis of the variance (ANOVA) of diffeent parameters was performed using the Genstat Discovery Edition 4 software. The Student Newman Keuls (SNK) test was used for to discriminate means for each parameters at 5% level.

Results:-

Tomato varieties grown in the Central Region of Togo
On all the fields visited, 7 varieties are produced, of which 6 in Sotouboua prefecture, 3 in Tchaoudjo prefecture, 2 in Tchamba prefecture and 7 in Blitta prefecture (Table 1). The results showed that there is more variety of tomato produced in Blitta especially in Adele (Kélébo and Gédèmè) than in other prefectures such as Tchamba. The fruits of these varieties are very fleshy and have an elongated shape easy to market.

Table 1: List of varieties grown in the Central Region of Togo

Prefectures	Tomato varieties grown
Sotouboua	Locale, Petomech, Roma, Tropimech, Platinium, Cobra
Tchaoudjo	Locale, Petomech, Tropimech
Tchamba	Locale, Petomech
Blitta	Locale, Petomech, Roma, Tropimech, Platinium, Cobra, Padma

Fungal diseases of tomato
Four major symptom types were observed on tomato plants in the fields: crown rots, white rots, early blight, *Fusarium* wilt.

Incidence of fungal diseases at field level
In most of the tomato fields visited, symptoms of fungal diseases including crown rot, white rot, *Alternaria* and *Fusarium* blight were observed. The incidence rate of these diseases at the field level varied from 0% to 100% with an average of 43.75%. The most common disease was the crown rot followed by *Fusarium* and *Alternaria* blight. The prefectures of Sotouboua and Tchamba recorded higher disease incidence rate (Table 2).

Incidence of fungal diseases at plant level
At the plant level, the fungal disease incidence varied from 0% to 15% with an average of 7.5% for crown rot and 1.5% for white rot. The highest rate (15%) was obtained at Nima in the prefecture of Sotouboua. The average incidence rates, 5% to 10%, were recorded in the localities of Nigbaoudè (Tchaoudjo), CECO (Sotouboua) and the localities of Tchamba. The lowest disease incidence (less than 5% on average) was observed in the localities of Gédémé and Kélébo in the prefecture of Blitta (Table 3).

Table 2: Fungal disease incidence (%) of tomato at field level

Prefectures	Localities	Fungal diseases*			
		crown rot	White rot	Early blight	*Fusarium* wilt
Sotouboua	Tchouloum Anie	100a	50b	50b	100a
	CECO	100a	100a	50b	100a
	Djamde Mono	100a	100a	0c	100a
	Nima	100a	50b	0c	100a
Tchaoudjo	Sabaringade	50b	0c	0c	0c
	Nigbaoude 1	50b	0c	0c	100a
	Nigbaoude 2	50b	0c	0c	100a
	Lomnava	50b	0c	0c	0c
Tchamba	Alibi 1	100a	0c	100a	0c
	Tchamba	100a	0c	100a	0c
	Afemboussou	100a	0c	100a	0c
	Ata Cope	100a	0c	0c	0c
Blitta	Gedeme	50b	0c	0c	100a
	Kelebo	50b	0c	0c	100a
	Tchifama	50b	0c	0c	50b
	Pagala	50b	0c	0c	50b
Average		75ab	18.8c	25bc	56.2b

Incidence at field level: PPDS = 25.28; * = values in the column followed by the same letter are not significantly diffrent at 5% Level.

Table 3: Fungal disease incidence (%) of tomato at plant level

Prefectures	Localities	Fungal diseases*			
		Crown rot	White rot	Early blight	*Fusarium* wilt
Sotouboua	Tchouloum	5d	5d	5d	5d
	CECO	10b	10b	5d	5d
	Djamde Mono	5d	5d	0 e	5d
	Nima	15a	5d	0 e	5d
Tchaoudjo	Sabaringade	5d	0e	0 e	0 e
	Nigbaoude 1	5d	0e	0 e	10b
	Nigbaoude 2	5d	0 e	0 e	10b
	Lomnava	5d	0 e	0 e	0 e
Tchamba	Alibi 1	10b	0 e	10b	0 e
	Tchamba	10b	0 e	10b	0 e
	Afemboussou	10b	0 e	10b	0 e
	Ata Cope	10b	0 e	0 e	0 e
Blitta	Gedeme	5d	0 e	0 e	10b
	Kelebo	5d	0 e	0 e	10b
	Tchifama	10b	0 e	0 e	5d
	Pagala	5d	0 e	0 e	5d
Average		7.5c	1.5 e	2.5e	4.38d

Incidence at plan level: PPDS = 2.17; * = values in the column followed by the same letter are not significantly diffrent at 5% Level.

Results of fungal identification

A total of 165 over 263 samples were positive for fungal isolation. The prefectures of Tchamba and Tchaoudjo recorded high isolation rates of 80.95% and 76%, respectively (Table 4).

Macroscopic and microscopic observations allowed the identification and characterization of the isolated pathogens. *Crown rot disease caused by Sclerotium rolfsii:* Four days after incubation, cottony white mycelium developped on PDA medium. From this culture, many small reddish brown spherical sclerotia dvelopped from the white mycelim by 10 days (Figure 1a). The analysis of microscopic characters through the observation of sclerotia and mycelium under a high-magnification photonic microscope revealed that the fungal was *S. rolfsii* Sacc.*White rot disease caused by*

Sclerotinia sclerotiorum. Four days after incubation, cottony white mycelium developped on PDA medium. Unlike the first culture of *S. rolfsii*, black nodules or sclerotia characteristic to the fungus *S. sclerotiorum* developped from the culture by 10 days (Fugure 1b)

Table 4: Rate of fugal isolation from samples

Prefectures	Blitta	Sotouboua	Tchaoudjo	Tchamba	Total
Samples tested	98	119	25	21	263
Positives samples	64	65	19	17	165
Rate (%)	65.30	54.62	76	80.95	62.73

a)  b) 

Figure 1: ***a)Pure culture of*** **S. rolfsii** ***with redish brown sclerotia on PDA ; b) Pure culture of*** **S. sclerotiorum** ***with black sclerotia on PDA (Bamazi, 2021)***

Tomato early blight caused by Alternaria solani: In the fields, irregular black spots turning on lesions (blight) symptoms with concentric rings were observed on stems, leaves and fruits. The typical and very characteristic symptoms were those on stems that are sunken and oval-shaped. The fungus appeared white-gray on PDA and turned darkly by the time (Figure 2).

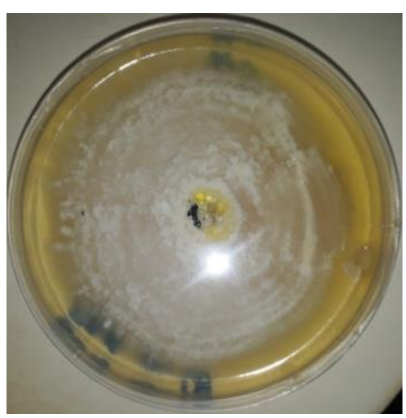

Figure 2: ***Pure culture of*** **A. solani** ***with black sclerotia on PDA (Bamazi, 2021)***

Tomato alternariosis is a disease that attacks all parts of the plant (leaves, stems and fruits) and all tomato plants regardless of their stage of development (vegetative state, flowering, fruiting and maturing). Observation of the mycelium under a photonic microscope showed several spherical and segmented spores.
The *Fusrium* wilt caused by *F. oxysporum* fungus was identified by the isolation of plant samples with wilt symptoms other than bacterial once. The culture was and a dense white mycelium on PDA which turned to purple by the time. (Figure 3).

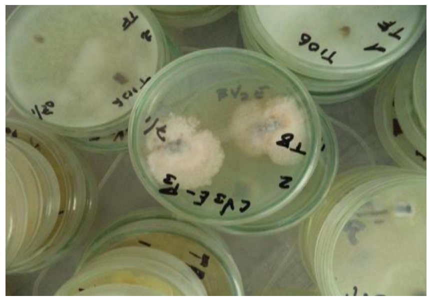

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Figure 3: ***Pure culture of* F. oxysporum *on PDA (Bamazi, 2021)***

Bacterial diseases

Bacterial wilt of tomato

On all the localities visited, symptoms of the whole tomato plant wilting but still green were observed (Figure 4). This symptom is typical to bacterial infection, especially that of *R. solanacearum*. The disease was observed also on several Solanaceae such as gboma, pepper).

Figure 41: ***Bacterial wilt of tomato plant at Kélébo (Bamazi, 2021)***

Bacterial spots of tomato

Small circular dark brown to black spots or water-soaked spots were observed, especially on the underside of the leaves (Figure 5). These spots are randomly distributed on the leaves and extend to fuse and form a large necrosis. These symptoms are characteristic to *Pseudomonas syringae* pv. *tomato*. These symptoms were found in the localities of Nigbaoudè 1 (Tchaoudjo) and Kélébo (Blitta).

Incidence of bacterial diseases

Incidence of bacterial wilt of tomatoat field level**:** Bacterial wilt was found in all the localities visited (incidence 100%). The results revealed a wide spraid of bacterial wilt in the surveyed area.
Incidence of bacterial wilt of tomato at plant level:The **i**ncidence varied from 10% to 43.33%. The localitites of CECO in the prefecture of Sotouboua, Tchifama and Gédemeè in the prefecture of Blitta recorded the highest plant level incidence with 43.33% and 41.67%, respectively. The lowest disease incidence of 10% was observed in the prefectures of Tchamba and Tchaoudjo (Table 5).

Figure 5: ***Bacterial leaf spot of tomato (Bamazi, 2021)***

*Tableau 5:****Incidence of bacterial wilt of tomato at plant level***

Prefectures	Localities	Incidence at plant level (%)
Sotouboua	Tchouloum_Anié	21.67 ± 1.67c
	CECO	43.33 ± 3.33e
	Djamdè-Mono	18.33 ± 1.67bc
	Nima	23.33 ± 1.60 c
Tchaoudjo	Sabaringadè	11.67 ± 1.66a
	Nigbaoudè 1	10.00 ± 0.00a
	Nigbaoudè 2	10.00 ± 0.00a
	Lomnava	10.00 ± 0.00a
Tchamba	Alibi 1	15.00 ± 0.00ab
	Tchamba	10.00 ± 0.00a
	Afemboussou	10.00 ± 0.00a
	Ata-copé	11.67 ± 1.66a
Blitta	Gédémè	41.67 ± 3.33e
	Kélébo	28.33 ± 1.66d
	Tchifama	41.67 ± 1.67e
	Pagala village	28.33 ± 1.67d
Moyenne		20.94 ±1.77

Incidence at plan level: PPDS = : 4. 801; * = values in the column followed by the same letter are not significantly diffrent at 5% Level.

Incidence of bacterial spots of tomato at field and plant level

Bacterial spots were of observed in only two localities (Nigbaoudè 1 and Nigbaoudè 2) in the prefecture of Tchaoudjo over all prefectures surveyed. The disease incidence was 100% at fields level in both of the localities, and was 5% and 2% at plant level in the localities of Nigbaoudè 1 and Nigbaoudè 2, respectively.

Results of streaming testing

The water glass test was positive in most of the communities visited. The sites in which the test was clearly positive are Kélébo, Gédémè in the prefecture of Blitta, Tchouloum, Djamdè-Mono, CECO in the prefecture of Sotouboua, Sabaringadè and Nigbaoudè 2 in the prefecture of Tchaoudjo and Tchamba (Table 6, Figure 6).

Table 6: Results of streaming testing for *R. solanacearum*

Prefectures	Sites	Result (+/-)
Sotouboua	Tchouloum_Anié	+
	CECO	+
	Djamdè-Mono	+
	Nima	-
Tchaoudjo	Sabaringadè	+
	Nigbaoudè 1	-
	Nigbaoudè 2	+
	Lomnava	-
Tchamba	Alibi 1	-
	Tchamba	+
	Afemboussou	-
	Ata-copé	-
Blitta	Gédémè	+
	Kélébo	+
	Tchifama	-
	Pagala village	-

The Figure 6 shows obviously the positive result of the plass water or streaming testing, by the bacterial flows (viscous, whitish liquid) perceptible through the transparent glass.

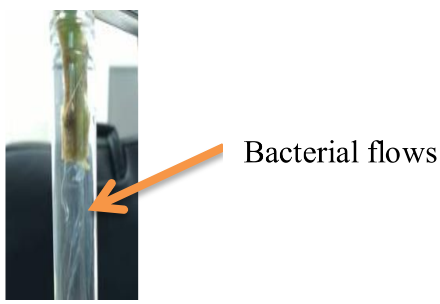


Figure 6: ***Result of positive streaming testing (Bamazi, 2021)***

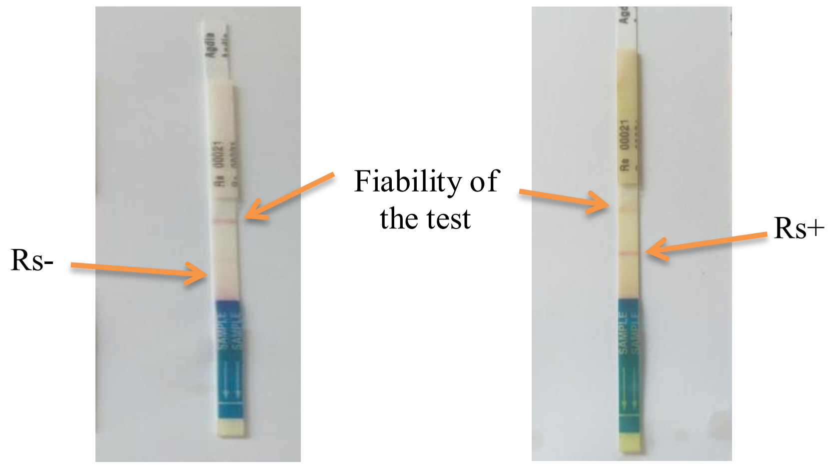
Over the 263 samples collected and tested with a glass of water, 205 were positive for *R. solanacearum*, giving a rate of 77.94%. The highest rate was obtained in Tchaoudjo, followed by Blitta which had a very large number of positive streaming samples (Table 7).

Results of Rs-specific immunstrip testing

As soon as the kit rod is properly immersed in the grinding of the test sample mixed with the buffer, the appearance of the top bar (upper line) indicates that the test is viable. In addition, when a second bar appears (bottom line) (Figure 7-b), this indicates that the test is positive and therefore reveals the presence of *R. solanacearum* in the corresponding sample. Otherwise, the test is negative, thus no presence of *R. solanacearum* in the sample.

Table 7: ***Results of streaming testing in the prefectures***

Préfectures	Blitta	Sotouboua	Tchaoudjo	Tchamba	Total
Samples tested	98	119	25	21	263
Positive samples	90	75	25	15	205
Rate (%)	91.83	63.02	100	71.42	77.94



(a) **(b)**

Figure 7 : ***Results of* Rs*-specific immunostrip test***
(a) Rs- = absence of bacteria; (b) Rs + = Presence of bcteria.

The results of the immunostrip testing varied from 20% to 100% over the visited localities. The sites of Nigbaoudè 2, Afemboussou, Pagala, Tchifama and Alibi recorded rates ranging from 20% to 50%, while Gédemeè, Kélébo, Djamdè-Mono and Nima had rates ranging from 50% to 80% contrary to the sites of Tchouloum and CECO which recorded rates of 100%. At the prefecture level, the average positive test were 38.89% in Tchaoudjo, 41.67% in Tchamba, 63.89% in Blitta and 88.89% in Sotouboua. From a total of 144 tomato samples tested with the immunostrip method, 84 (58.33%) were positive to .*R solanacearum*, the highest positive sample recorded in the prefecture of Sotouboua followed by that of Blitta (Table 8).

Table 8: ***Results of* Rs*-specific immunostrip testing***

Prefectures	Number of samples	*Rs* positive samples	Rate (%)
Sotouboua	36	32	88.89
Tchaoudjo	36	14	38.89
Tchamba	36	15	41.67
Blitta	36	23	63.89
Total	144	84	58.33

Results of bacteria isolation

After isolation on NA and SMSA, mucoid, beige colonies with a purple to red color in the centre characteristic to *R. solanacearum* were observed on the medium. From the 84 samples positive at immunostrip testing, 45 (53.57%)

yielded *R. solanacearum*, including 12 isolates from Blitta, 22 from Sotouboua, 5 from Tchaoudjo and 6 from Tchamba. (Table 9).

*Table 9: **Results of tests and** **R. solanacearum** **isolation***

Localities	TS	Stream+	Immuno +	*Rs* Isolates	NL	EL
Tchouloum_Anie	14	9	9	9	8.540300	0.953574
Sotouboua Ceco	47	9	9	9	8.564600	0.964759
Djamde-Mono	41	9	9	2	8.585928	1.247238
Nima	17	9	9	2	8.709822	1.038777
Sabaringade	11	9	9	2	8.996942	1.333252
Nigbaoude 1	7	9	9	1	8.968651	1.353807
Nigbaoude 2	3	9	9	1	8.970250	1.348426
Lomnava	4	9	9	1	9.009640	1.122500
Alibi 1	7	9	4	2	8.947897	1.417715
Tchamba	5	9	3	1	9.033333	1.416660
Afemboussou	4	9	3	1	9.129160	1.520800
Ata-Cope	5	9	5	2	8.913902	1.418092
Gedeme	17	9	7	3	8.234152	0.697053
Kelebo	51	9	8	4	8.218284	0.809689
Tchifama	15	9	5	3	8.191663	0.8088772
Pagala	15	9	3	2	8.208191	0.8864854
Total	**263**	**144**	**84**	**45**	**-**	**-**

TS = total samples collected; Stream+ = streaming positive test; Immuno+ = Immunostrip posotive test; *Rs* isolates = *R. solanacearum* isolates obtained; NL = North Latitude; EL = East Longitude.

Results of PCR analysis of bacteria isolated from wilt samples

The molecular analysis carried out by Kunwar and Bamazi et al. (2022) showed that all samples migrated to phylotype I, similar to the control sample GMI1000. The genomic analyses revealed revealed that these srtains belong to *R. solanacearum* spieces "phylotype I - sequevar 17".

Results of pathogenicity testing with R solanacearum strains

All strains of *R solanacearum* from the four prefectures caused general wilting of tomato plants after inoculation. Among these 4 strains, the T18 strain of the CECO locality in the prefecture of Sotouboua was the most virulent one. After one week, it caused wilting of all the inoculated plants (Figure 8).

Types of pesticides used by vegetable producers

The surveys identified 10 synthetic chemical pesticides from different trade names including 6 insecticides (60%), 1 acaricide (10%) and 3 fungicides (30%). These phytosanitary products are of different formulations, either liquid in Emulsifiable Concentrate (EC) or Soluble Concentrate (SL) and mainly insecticides and acaricides or powdered especially powder formulated fungicides (WP). Among all these pesticides in the studied area, no biopesticide was found. The K-Optimal was the most frequently used pesticide in the prefectures of Blitta, Sotouboua and Tchamba with 71.15% of the farmers investigated. Lambda Super was the second most used insecticide with 57.69% of the farmers in the prefectures of Blitta, Sotouboua and Tchaoudjo. In the prefecture of Sotouboua, acaricide Acarius was used by only two producers (3.85%), while Mancozan (30.77% of the farmers) was one of the most commonly used fungicides in the prefectures of Blitta and Sotouboua (Table 10).

Figure 8: ***Wilting of tomato plant after inoculation with* Rs *suspension.***

Table 10: ***Types of pesticides used by producers***

	Insecticides							Ac	Fongicide		
Préfecture	Nr	K*	Ld*	Em*.	Py*	Ins**	L**	Ar*	Man*	O**	Iv*
Sotouboua	20	11	6	2	4	0	2	2	9	4	2
Tchaoudjo	6	1	5	0	0	0	0	0	0	0	0
Tchamba	8	8	0	1	0	0	0	0	0	0	0
Blitta	18	18	18	1	0	7	0	0	6	0	0
Region	52	37	30	4	4	7	2	2	16	4	4
Rate(%)	100	71.1	57.6	7.6	7.69	13.4	3.85	3.85	30.77	7.69	7.69

* : Produits homologués ; ** : Produits non homologués ; Nr = number ; K: K-optimal; Ld: Lambda cyhalothrine; Em: Emacot; Py: Pyriforce; Ins: Inscetor; L: Lacare; Ac: Acaride; Ar: Acarius; Man: Mancozan; OKM: O.K.MIL; Iv: Ivory.

An analysis of these pesticides revealed 70% were from the approuved list of pesticides in Togo (DPV, 2021). However, 30% of the pesticides with various trade names were not authorized/approuved for use as plant protection products in the study area are then not registered. Figures 9 and 10 show the approuved and none approuved pesticides, respectively found in the Central Region of Togo.

Relationship between pesticides used and targeted pests

The results indicated that 92% of the farmers used synthetic chemical pesticides to control tomato bioaggressors. Most of them (55.77%) used exclusively insecticides, 36.54% combined insecticides with some fungicides, while 7.69% did not use chemical pesticides in the fields. All the producers interviewed treat the fields as soon as there is an attack without identifying the bioaggressor (fungal, bacterial, viral disease, attack of insects, mites, nematodes or abiotic factors). Thus, the pathogens were not offenly targeted by the treatments (Table 11). The identification must be prior to phytosanitary treatments on targets pests.

Table 11: ***Farmers practices in the use of pesticides***

	Pesticides	Target	None target	Rate (%)
Insecticides	K.optimal	Flying and crawling insects	Treatment on any symptoms (insect damages, mites, abiotic biotic diseases)	71.15
	Lamda super	Insects		57.69
	Emacot	Worms		7.69
	Pyriforce	Insectes		7.69
	Insector	Seed treatment		13.46
	Lacare	Insects		3.85
Acaricide	Acarius	Acariens	Tout dégât	3.85
Fongicide	Mancozan	Fungal diseases of vegetables	Insects treatment	30.77
	O.K.MIL	Brown rot of cocoa pods	Vegetables	7.69
	Ivory	Preventive action	Curative action	7.69

Figure 9: ***Products from approuved list of pesticides (Bamazi, 2021)***

*Figure 10:****Products out of the approuved list of pesticides (Bamazi, 2021)***

Respect of products doses and preharvest interval

The specific pesticides are most of the time more expensive in the markets/shops, thus growers prefere buying low cost synthetic pesticides that are not recommended or not approuved for use in controlling pests in vegetables production. Besides, most of the farmers did not respect the instructions for use; also the lack of precision tools for measuring products contributes to no respect of the label requirements. For this, farmers used pesticides bottle caps, empty tomato boxes, spoons, etc. as measurement units. In the Central Region and average of 73.07% of the producers did not respect the doses of the pesticides recommended for treatment. They were 87.5% in the prefecture of Tchamba, 65% in the prefecture of Sotouboua (Table 12). Regarding the Pre-harvest Period or pre-harvest interval – PHI - (number of days to respect between the last phytosanitary treatment and the harvest of the product), the results revealed an average of 9.96% of farmers not respecting that interval (Table 12).

Table 12: ***Proportions of farmers with no respect of intructions of pesticides use***

Prefectures	Localities	No respect (%)	
		DT	PHI
Sotouboua	Tchouloum	100	0
	CECO	77.77	0
	Djamde M.	50	0
	Nima	50	25
Tchaoudjo	Sabaringade	50	0
	Nigbaoude 1	50	50
	Nigbaoude 2	100	0
	Lomnava	100	0
Tchamba	Alibi 1	100	0
	Tchamba	100	50
	Afemboussou	100	100
	Ata Cope	66.66	0
Blitta	Gedeme	83.33	0
	Kelebo	71.42	0
	Tchifama	66.66	33.33
	Pagala	100	0
Region level means		73.07	9.96

DT = Dose of treattment; PHI = Pre-harvest interval.

Protective measures

The results revealed that none of the farmers surveyed did not have full personal protective equipment (PPE). Only 13.46% have either masks, gloves or boots. Self protection and sanitary measures are most of the time neglected by the vegetable producers. According to them, there is no risk for the applicant of the treatment, so no need to wearing protective equipment; controlling wind direction is enougth to equive risk. Also, the PPE is unavailability on the market or, when available, it is too expensive for them. This can lead to immediate or long-term risks they face in the use of pesticides.

Packaging management

Most of producers (80%) abandon the empty packaging pouches of pesticides such as Mancozan 80 WP, Ivory 80WP on sites after treatment even at their resting places or at the place of preparation of the product. In the case of containers for K-Optimal EC, Lambda-super EC, A48 EC CARIUS18EC, Pyriforce 48 EC, 95% of the producers recycle them after use, as drinking water containers, local drink or condiment boxes (salt, pepper, oil, etc.). Less than 20% of farmers burn the containers, bury them or hang empty pesticide packages on trees. This form of management is due to the fact that most farmers underestimate the risks incurred.

Discussion:-

The symptoms of fungal diseases observed during site prospecting are identical to those described in Benin by Sikirou et al., (2015). These symptoms are leaf spots, organ decay and plant wilt. To better control these diseases, a study on integrated pest management in vegetable production was carried out in Benin in 2010 and a guide for extension agents in West Africa was produced (James et al., 2010). This study inventoried in Benin, the same fungal diseases that our study diagnosed in the Central Region in Togo. The fungus *S. rolfsii* is present in soil and on plant debris. Contaminated plants exhibit yellowing and progressive leaf wilt (James et al., 2010). One study also reported the presence of this pathogen in Benin, which is primarily identified by the white layer of mycelium at the base of contaminated stems (Sikirou et al., 2015). This thick white layer of mycelium has several small hard spherical bodies, known as sclerotia.

Fungal incidence rates at the field level and low to moderate incidence at plant level were observed for the différence diseases found. This result is related to studies by Sikirou et al., (2009) in Benin, where the incidence of the different diseases did not exceed 30%. These authors found *Fusarium* wilt but also the collar rot caused by *S. rolfsii*. The late fungus is present in the soil and infect host plants therefrom and the temperatures of 25 to 35°C are welfair for its development (Blancard et al. 2009).

The incidence of fungal diseases is higher than that of bacterial diseases. This difference could be explained by the fact that fungal diseases are more diversified and their modes of transmission more favorable than bacterial diseases in the Central Region. This incidence rate is higher in the prefectures of Blitta (wetland area, Adélé) than in the prefectures of Tchamba, Tchaoudjo and Sotouboua (savannah area). This can be explained by the location of sites near rivers around which the moisture content is higher (above 65%) and the large amount of runoff.

The disease of bacterial wilt of tomato has been identified in all prefectures of the Central Region. The incidence rates both at field and plant level were higher in the Central Region of Togo. This findings are related to the wok carried out by Kunwar and Bamazi et al. (2021), who reported bacterial wilt of tomato incidence of up to 50% in most fields in Sotouboua. The symptoms observed in this study are were similar to those previously described by Sikirou et al., (2009); Ayissah et al. (2019), Bamazi et al. (2019); Kunwar and Bamazi et al. (2021). The disease was found in all the four prefectures surveyed. Based on scientific and economic importance, *R. solanacearum* is the second most important pathogen among the best ten worldwide (Balamurugan et al., 2020; Xue et al., 2020). *R. solanacearum* is present in West Africa (Ailloud, 2015), and was reported in Benin (Sikirou et al., 2009), Ghana (Subedi et al., 2014), Burkina Faso (Son et al., 2017) and Togo (Kunwar and Bamazi et al., 2021).

The disease was found in five of the nine agroecological zones of Benin including the central region of the country (Sikirou et al., 2017) located in the same latitudes as the central region of Togo, the study area. Bacterial wilt has been identified to be more severe in the central region of Benin than in other locations in the country (Sikirou et al., 2017). Togo and Benin being two neighbouring countries and sharing almost the same agro-ecological conditions from north to south, the impacts of this disease have been more noticeable in the prefectures of Blitta and Sotouboua than in the other prefectures that are located in the north of the central region in Togo. Although the disease is present in all locations of the 4 prefectures visited in Togo, its incidence is 43% compared to 71% in Benin in (Sikirou et al., 2017). The results of 100% incidence of diseases compared to fields in the Central Region indicate that soils, watering water, seeds, work equipment, are infected with *R. solanacearum.* This bacterium has the ability to survive and disseminate through plant material and water, thus constitute a bank of inoculum offseason (Kumar et al., 2017). From these inoculum, the bacteria can spread from one diseased field to another through several factors/means including watering, rain, wind and biotic agents. Other sources of contamination are the exchange of infected seeds of planting material, equipments/tools (Shahbaz et al., 2015) as is the case in the Central Region of Togo. Infected semi-aquatic weeds can also play a role in disseminating the pathogen by gradually releasing root bacteria into rivers (Champoiseau and Momol, 2008). The Anié and Tchoulom rivers in Sotouboua, Kadoulissi in Nima, Kapété in Alibi 1, Kélékou in Gédémè, Koung in Kélébo, Kpantchrou in Tchifama, Bambri in Nigbaoudè and the Mono River in Djamdè-Mono are the sources of watering water (Bamazi et al., 2019) and therefore possible sources of contamination of crops.The Anié and Tchoulom rivers in Sotouboua, Kadoulissi in Nima, Kapété in Alibi 1, Kélékou in Gédémè, Koung in Kélébo, Kpantchrou in Tchifama, Bambri in Nigbaoudè and the Mono River in Djamdè-Mono are the sources water used for watering (Bamazi et al., 2019) and therefore possible sources of contamination of crops. Beyond this situation, the poor cultivation practices used by producers as well as the lack of rotation are also a source of spread of the disease.

Bacterial wilt of tomato was observed at all stages of the plant development, but the disease became very important at the fruiting stage leading to total yield losses of tomato. Once present in a field, beside tomato, other Solanaceae crops such as potato (Hayward, 1991; Elphinstone, 2005; Mansfield et al. 2012), pepper and eggplant. (Sikirou et al., 2017),pepper and gboma (*Solanum macrocarpon*) (Kunwar and Bamazi et al. 2021) can't be produced without suitable control measures.

In addition, it should be added that the proliferation of bacterial diseases is favored by the excessive use of nitrogen fertilizers (Paret et al., 2011) and this is the case in Adele compared to the northern zone of the Central Region. Our work on the inventory of tomato diseases in the Central Region of Togo leads to the same conclusions as the studies of Ayisah et al. (2019) in Togo which suspected the presence of *R. solanacearum* in tomato fields; Sikirou et al. (2009) Benin and James et al. (2010) at ITTA in Benin. From these studies, it appears that bacteria such as *R. solanacearum* and fungi are present on farms and responsible for tomato diseases in the Central Region of Togo.

The evaluation of the phytosanitary practices of producers in the Central Region of Togo revealed that more than 30% were trained and supported by the extension workers, especially from ICAT, compared to 14% in Togo (Kanda et al., 2013) and 9% in Burkina Faso (Son et al., 2017). The training taken by the producers may have contributed to the achievement of 70% of them using pesticides from the approved list. However, Kanda et al. (2013) reported that 90% of the pesticides used were from local markets without any guarantee of conformity and quality. The pesticides found did not belong to those used for cotton pests control as it was the case in Burkina Faso (Son et al., 2017). The use of synthetic pesticides by producers is real in the Central Region in the present study as was also reported some years ago (Kanda et al., 2013; Mondédji et al., 2014). Insecticides of the Pyrethrenoides families are widely used in the Central Region in Togo. Pyrethroid-based insecticides such as lambda-cyhalothrin and deltametrin are the pesticides widely (Naré et al., 2015; Son et al. 2017) on vegetables in Benin (Lawson et al., 2017), in Côte d'Ivoire (Doumbia and Kwadjo, 2009) and in Senegal (Diop, 2013). The harmful effects of pesticides are not really known by most of producers. However, the impacts of pesticides on human, animal and environmental life and bioaggressor resistance to insecticides have been largely documented (Assogba-Komlan et al., 2007; Kanda et al., 2013 ; Mondédji et al., 2014 ; Agboyi et al., 2016; Son et al., 2017). This led to environmental pollution resulting from both overdose effects and poor packaging management of the chemical products. Moreover, pesticides uses are poorly targeted to bioaggressors/pests leading to yields and economic losses.

The present results revealed that more than 70% of the producers did not respect the products doses recommended for treatment. These findings support the reports of Kombieni and Akoda (2017), Snelder et al. (2008), Doumbia and Kwadjo. (2009) and Ahouangninou et al. (2011). The no use of protective equipment found in the present study is a general situation in several African countries as reported by Kombieni and Akoda (2017) in Togo, Doumbia and Kwadjo crops. (2009) in Côte d'Ivoire.

After use of pesticides, packaging/conteners of pesticides were not properly recycled or were abandon in the nature resulting to environmental pollution. Thiam and Sagna (2009), Sougnabe et al. (2009) and Gomgnimbou et al. (2009) showed that packaging/conteners of pesticides were never recycled or returned to distributors. Kombieni and Akoda (2017), found tht more than 65% of the producers abandon the packaging/conteners in the nature, leading to environment pollution as have been reported also in Senegal by Cissé et al. (2003), in Nigeria by Akan et al. (2013).

Conclusion:-

This study established the status of tomato major diseases in the Central Region of Togo, their incidence and the idification of pathogens. The fungal pathogens of tomato included *S. rolfsii, S. sclerotiorum, A. solani, F. oxysporum*, whereas the bacterial pathogens were *R. solanacearum* and *Pseudomonas syringae* pv. *tomato*. Wilts are more severe in areas with high relative humidity. *R. solanacearum* is a serious constrains to many Solanaceae crops production.Based on the present results, investigations on tomato losses due to *R. solanacearum* infection need to be carried out and suitable control strategies must be developped for better managment of the pathology.The unsuitable use and marketing of pesticides constitutes risks to human as welle s environment. Many wrong or improper phytosanitary practices, including the unsupported use of pesticides, packaging management, dose of products for treatments, product application intervals and times of treatments, preharvest intervail or delay were identified. It is obvious that the environment components - air, soil, water - of the surveyed area might be contaminated by synthetic chemical pesticides and the consequences are obviously the negative impact on human health. Thus, special attention mus should be given for developping suitable control strategies to avoid yield losses for producers.

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