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RESEARCH ARTICLE

STRUCTURE OF NEMATODE COMMUNITIES ASSOCIATED WITH COWPEA (VIGNA UNGUCULATA, (L) WALP) AS A FUNCTION OF THE PHYSICOCHEMICAL CHARACTERISTICS OF THE SOIL IN THE MARKET GARDENING AREA OF SOTUBA (MALI)

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Abstract

A nematologicalstudy on cowpea (Vignaunguculata, (L) Walp) wascarried out in 2022 during the rainyseason on the experimental plots of the Sotubaresearch station in Mali. The aim of the studywas to identifyparasiticnematodesassociatedwiththislegume and to assess the influence of soilfactors on the nematofauna. Nematodeswereextractedusing the modified Baermann technique. From the observation of soilsamples, 6 genera of phytonematodeswereidentified:These are Pratylenchus, Tylenchorhynchus, Helicotylenchus, Scutellonema, Meloidogyne and Tylenchus. The generaHelicotylenchus and Tylenchuswere the most important in terms of density and representapproximately 55% of the number of nematodesencountered. The pedologicalanalysisincludingchemical and granulometricparametersshowed an acidictendency of the soil, with a silty-sandy structure. The porosity of suchsoilcouldbe the basis of the distribution of nematodeswhichwerefound to be more abundant in the third plot whichcontains the highest rate of sand 69%. Among the nematodesidentifiedwerespeciesthat are harmful to cowpea, includingMeloidogyne. This study shows the need for phytosanitary monitoring to preventextremeoutbreaksthatcould cause a significant drop in cowpeayields.

Introduction:-

Belonging to the Fabaceaefamily and the genusVigna, cowpea (Vignaunguculata, (L) Walp) is native to tropical Africa, whereseveralvarieties are cultivated for theirseeds. It wasdomesticated in ancient times, dating back to around 1500−1000 J.-C. in South Asia and around 300 earlierJ.-C. in the Mediterranean basin [1]. The French vernacularname niébé, is a Wolof termwhose use has becomewidespread in West Africa. Cowpeais the main foodlegume in tropical Africa, providing an economical source of very good qualityprotein. Young leaves and immature pods are evenconsumed as vegetables[2]. From an agronomic point of view, cowpeaalsoensures good soilfertilitythroughitsability to fix atmosphericnitrogen[3]. The speciesiscultivated in tropical and subtropical regions, particularly in sub-SaharanAfrica, whichaccounts for almost all of the world's production [3]. Annual global cowpea production isestimated at 6.4 millions tonnes from an area of approximately 12.7 million hectares. Sub-

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SaharanAfricaaloneaccounts for approximately 95% of this production, with more than 80% of Africa'ssharebeingproduced in West Africa[4]. [5] reports that Nigeria is the world'sleadingproducer of cowpea. In Mali, itis the main foodlegumecultivated for itsnutritional and economicinterest. In 2019, a production of 220,000 tonnes on an area of approximately 450,000 hectares wasachievedwith a yield of approximately 0.65 t/ha [6]. Cowpeais a plant welladapted to Sahelianclimatic conditions. Its short growing cycle ensures a certain food production, despite the rainfalldeficit in this area. In addition to climatichazards, soilpovertyconstitutes one of the major biophysicalconstraints to agricultural production in Sahelian countries [7]. This justifiestakingintoaccountseveralpotentiallimitingfactors of agricultural production, because no intervention alone can lead to a miracle response. Amongtheseconstraints, plant-parasiticnematodes have been reported as serious obstacles to cereal and vegetable production in differentregions of the world [8]. In sub-SaharanAfrica, almost all vegetablecropsexperienceyieldlosses due to plant-parasiticnematodes[9]. Theseyieldlosses can reach 25 to 40% in the absence of nematicidetreatment[10]. The general objective of thisstudywas to identifyphytonematodesassociatedwithcowpea cultivation. More specifically, itwas a question of determining the composition and importance of the nematodegeneraassociatedwithcowpea on the one hand and characterizing the soil structure in order to explain the linkbetween the nature of the soil and the development of nematode populations. Our hypothesisisthat in the presence of a host plant, soilcharacteristics influence the spatiotemporal distribution of parasiticnematode populations.

Materials and Methods:-

Study sites

This studywascarried out in the experimental plots of the Regional Center for Agricultural Research of Bamako in Mali located in Sotuba. Four cowpea plots of 400m^{2}eachwith the followinggeographicalcoordinates; 12°39’45’’N; 7°55’27’’W; 12°39’34’’N; 7°55’13’’W; 12°39’14’’N; 7°54’58’’W; 12°39’72’’N; 07°56'72’’W, wereconcerned by the study.

This site extendsbetween the 800 and 1000 mm/yearisohyet. It issubject to a Sudano-Sahelianclimatecharacterized by the existence of twoseasons: a long dry season (November to May) and a short rainyseason (June to October). Alluvial, hydromorphic, ferruginoussoilswith a sandy loam texture are generallyencountered in Sotubabecauseitis an ancient alluvial terrace of the Niger River [11].

Sampling

Soil data

The bio-pedologicalstudy of the soilswascarried out on the basis of cowpea cultivation on the site during the previousthreeyears. The composite samplesconsisting of soilfrom four plots were sent to the Soil Water Plant Laboratory (LABOSEP) in Sotubawhere a physicochemicalanalysiswascarried out to determine the differentgranulometric fractions: clay (Ag), fine silt (Lf), sand (Sb). Similarly, the carbon and nitrogen content (C and N) and assimilable phosphorusweredefined. To determine the soil texture of the different sites, the texture triangle wasused (Figure 1).

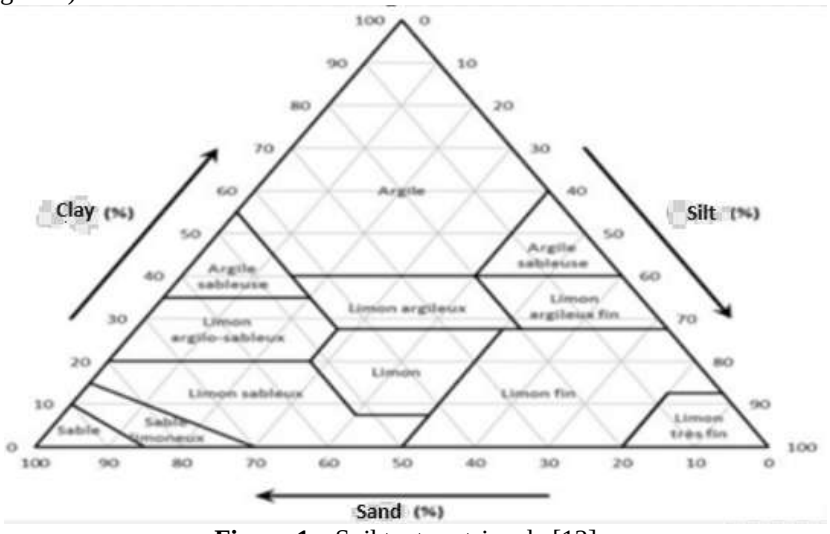

Figure 1:- Soil texture triangle [12].

Nematological data:

Sample collection:

Soilsamplesweretakenduring the rainyseason. This sampling wasdone in the rhizosphere of the cropsaccording to systematic sampling using a 6 cm diameterauger, and a depth of 25 cm. This type of sampling takesintoaccount the field as a whole and the aggregate distribution of nematodes. Afterscraping the surface part of the soilwith a daba, tensamples per plot weretaken at the level of 4 designated plots, i.e. 40 sampling points overall. The samplesfromeach plot wereseparatelyplaced in a smallbucket, cleared of large debris, and homogenized by hand. Fromthissoil, a sub-sample of 500g per plot wastaken and thenplaced in plastic bagsmarkedwith the name of the site, a sampling number, and the name of the source crop, all wellsealedwith a tie. The soilswerethencollected in a cooler and transported to the laboratory. In the laboratory, using a beaker, 100ml of soilfromeach plot weretakenfrom the four sub-samples for nematologicalanalysis. The remainingsoilisketptmoist for soil analyses.

Extraction of nematodesfrom the soil

To extract the nematodes, the modified Baermann technique wasused (Figure 2). 100 ml of soilfromeach plot waswrapped in a "kleenex" type paper, thenplaced on a 1 mm mesh PVC sieve. The wholeisplaced in a plate. Water wasthenadded to the sample in order to moistenitcompletely. After 48 hours, all the water in the container ispassedthrough a 38 µm meshsieve in order to keep a suspension of approximately 25 ml. Fromthis suspension, an aliquot of 5 ml isobservedunder a microscope at 40x magnification.

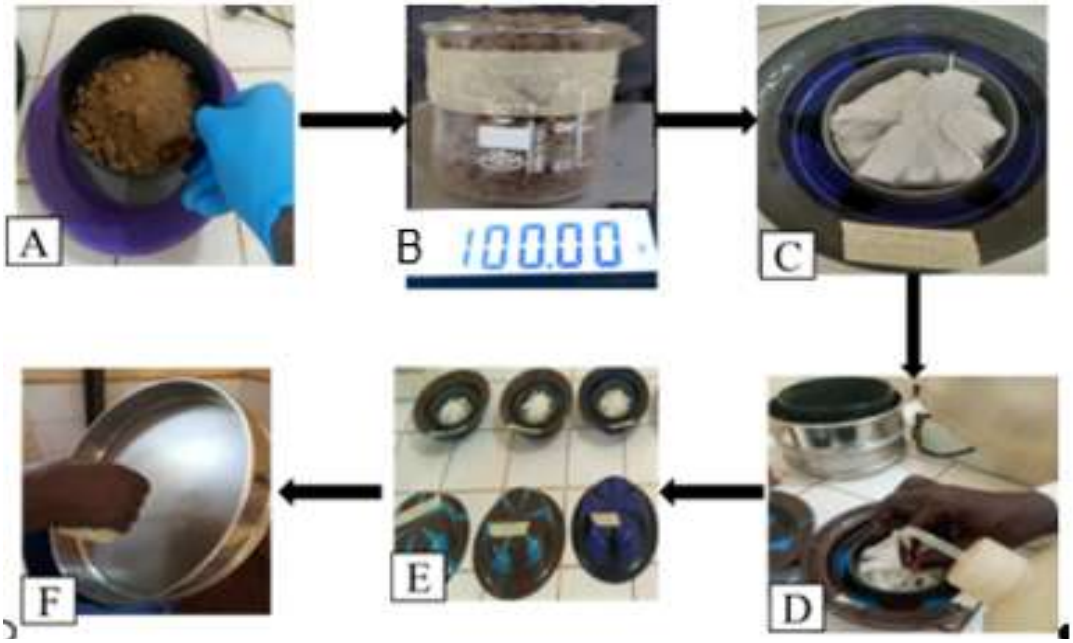

Figure 2:- The steps in the execution of the modified Baermann technique.

Nematode fixation

Nematodeswerefixed by immersion in a boiling FA solution composed of formalin 10ml; glacial aceticacid 1ml; distilled water 89 ml, [13]. The technique consists of adding the heated FA solution to the nematode suspension in equal volume in a smallvialwitha stopper. Nematodeswerecountedusing a stereoscope at 40x magnification.

Observations:-

The suspension washomogenizedusing a pipette with a bulb by aspirating and insufflating the solution, then an aliquot of 5 ml isplaced in a countingcellwith a gridbottom and observedunder a PARALUX invertedgallows microscope. The nematodes are observed and identified at the genuslevelthencounted. The results are reported as the number of nematodes in 100ml of soil.

Genericdetermination and counting of nematodes

This genericdetermination of phytonematodeswas made using a determination key [14] and discriminatingmorphologicalcharacters (Figure 3): the shape of the head; the size and shape of the stylet; the

position of the vulva; the covering of the intestine by the esophageal glands; the habitus; the shape of the tail, itsopacity[15].

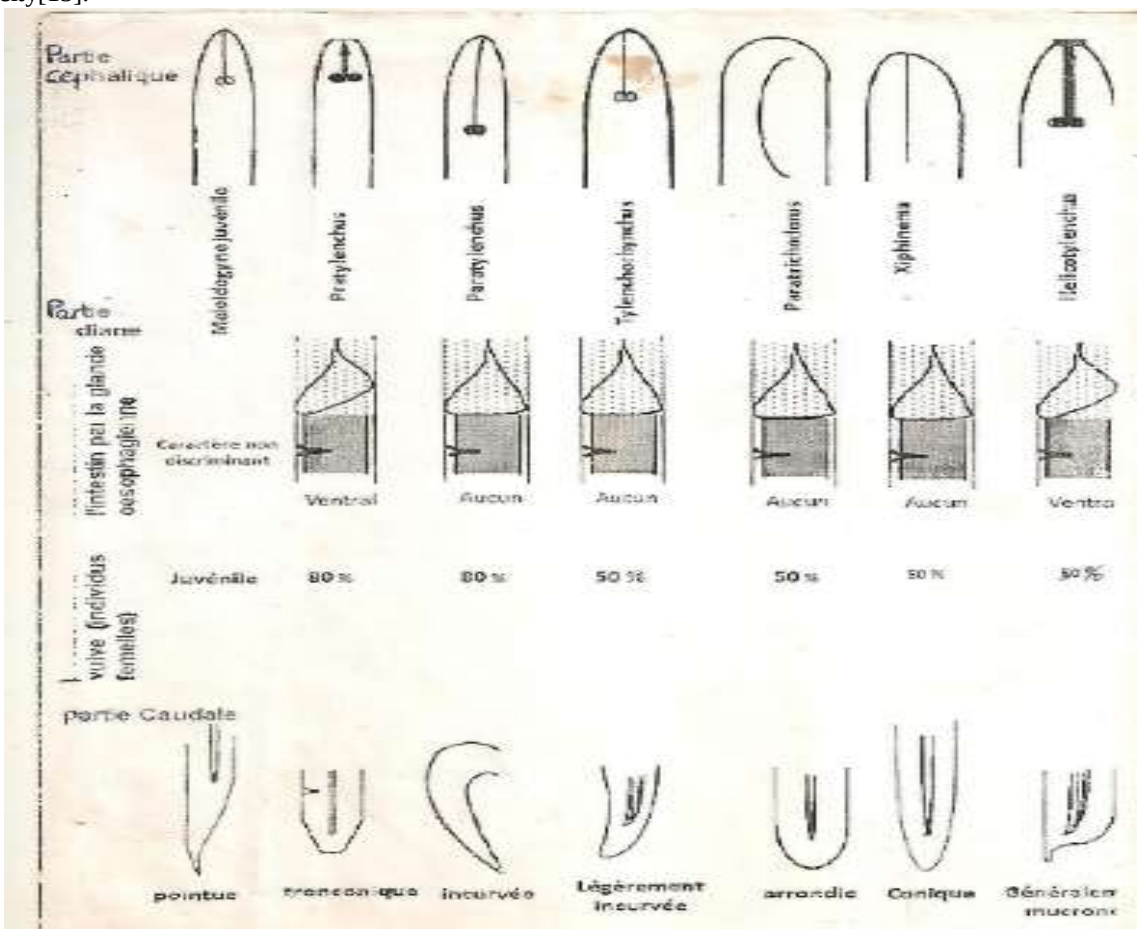

Figure 3:- Discriminatorymorphologicalcharacteristics[15].

Data processing

The enumeration of nematodegenerawascarried out under a stereoscope. From a 25 ml suspension (V), an aliquot solution of 5 ml (v) istaken and placed in a counting box with a gridbottom for counting. Afterenumeration, the population numberswereexpressed as the number of nematodes per dm^3 of soil (N/dm^3) according to the formula:

$$N = \frac{V}{v} X\ 5$$

The importance of eachgenus of nematodewasdetermined by calculating the density, abundance, frequency and occurrence by genus and by plot. An analysis of variance wascarried out to compare the means by genus. The frequency of occurrence (F) of a speciesis the ratio expressed as a percentage of the number of sampleswherethisspeciesisnoted to the total number of samplestaken:

$$F = \frac{Pn}{Pt} \times 100$$

F = frequency of occurrence of the species. Pn = total number of samplescontaining the speciesconsidered. Pt is the total number of samplestaken. There are three types of frequencies: constant species ($F \geq 50\%$), incidental species ($25\% < F < 50\%$), accidentalspecies ($F \leq 25\%$).The relative abundance (A) of a species corresponds to the ratio of the number of individuals of the samespecies to the total number of individuals of all speciescombined:

$$\mathbf{A} = \frac{Na}{Na + Nb + Nc + N...} \times 100$$

The data wereenteredinto an Excel 2016 spreadsheet for statistical analyses. An analysis of variance (anova), at the 5% threshold, wasperformed to discover possible differencesbetween the averagenematodedensities.

Results:-

Soil data

Table 1:- Rates of physicochemicalparameters of the plots studied.

Soil parameters		Plot 1	Plot 2	Plot 3	Plot 4
Chemicals	pH	6,1	6,2	6,3	6,1
	C	0,47	0,91	0,3	0,34
	N	0,04	0,02	0,01	0,02
	P ppm	13,405	12,67	23,38	17,92
Granulometric	Sand %> 0.05mm	28	61	69	48
	Fine silt % 0,05-0,002mm	59,5	30	23	38
	Clay % < 0,002mm	12,5	9	8	14
faunal parameters	Nematode densities	144	94	207	111
Nature of the soil		LF	LS	LS	L

Chemical parameters

Concerning pH, the analysis shows an acidictendency of the soilthroughout the field. The values foundwerebelow the neutralitythreshold (pH < 7). A comparison of these values shows thattheyvariedfrom 6.1 in the first and fourth plots to 6.3 in the third and 6.2 in the second (Table 1). As for organiccarbon, the contents alsovariedfrom one plot to another, the percentages oscillatebetween a maximum of 0.91 observed at plot 2 and a minimum of 0.30 in plot 3 (Table 1). Compared to organicnitrogen, the contents in the soilwererelativelylow. The chemicalanalysis shows a slight variation from one point to another, the minimum value was 0.01% noted in plot No. 3. The highest content wasobserved in the first plot with a value of 0.04%. For availablephosphorusexpressed in ppm, the highest value wasrecorded in the third plot with 23.38 ppm, followed by the fourthwith 17.92 ppm. 13.40 and 12.67 ppm wererespectively in the first and second plots (Table 1). Statisticalanalysis of chemicalparameters by plots showedthatthereis no significantdifferencebetween the plots (anova, p>5%).

Granulometricparameters

The granulometryincludesthreemeasuredparameters, sand (Sb) designatingparticleslargerthan 0.05 mm, fine silt (Lf) whichisformed by particlesbetween 0.05 and 0.002 mm and clay (Ag) composed of thosewhich are lessthan 0.002 mm on all the plots we observe (Table 1) an average of 51.50% sand, 37.63% fine silt and 10.88% clay. Sand is the most important followed by fine silt. The texture of the soilswasdeterminedusing the texture triangle. This determinationshowed a silty-sandysoil in plots n°2 and n°3, Silty in the first and fourth plots. Statisticalanalysis of the granulometricparameters by plot showedthattherewas no significantdifferencebetween the plots (anova, p>5%).

Faunalstudy

The nematologicalanalysisrevealed 6 genera on the perimeterwhich are: Meloidogyne, Pratylenchus, Helicotylenchus, Tylenchorhynchus, Scutellonema, and Tylenchus (Table 2). According to the mode of parasitism, the nematodes are dividedintothreetrophic groups: ectoparasites with four generawhich are: Tylenchus, Tylenchorhynchus, Helicotylenchus and Scutellonema, sedentary endoparasites represented by a genuswhichisMeloidogyne, and migratory endoparasites (Pratylenchus).

Table 2:- The genera of nematodesencountered.

Families	Genera	Trophic groups
Heteroderidae	Meloidogyne	Sedentary endoparasite
Pratylenchidae	Pratylenchus	Migratory endoparasite
Hoplolaimidae	Helicotylenchus	Ectoparasite
Belonolaimidae	Tylenchorhynchus	Ectoparasite
Hoplolaimidae	Scutellonema	Ectoparasite
Tylenchidae	Tylenchus	Ectoparasite

Importance of parasiticnematodes

The meandensities of nematodesvariedfrom one genus to another. The analysis of the results shows that the most important proliferationwasnoted for the genusHelicotylenchuswith 220 individuals/100ml of soil, followed by Tylenchuswith 138 individuals/100ml of soil. The lowestdensityisthat of the genusTylenchorhynchuswith 23 nematodes/100ml of soil (Figure 4). The statisticalanalysis of densities by genus shows thatthereis no significantdifferencebetween the densities (Anova, P = 0.09).

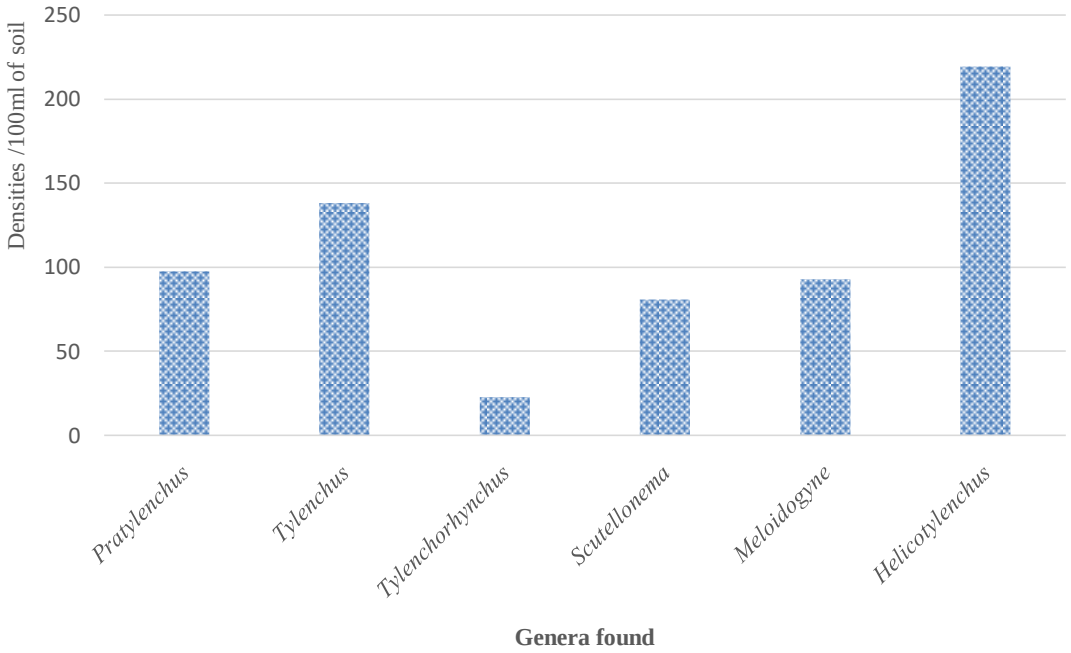

Figure 4:- Averagedensities of phytonematodes in 100ml of soil.

Structure of phytonematode populations:

Table 3:- Frequency of occurrence, abundance and densities of nematodesencountered. Legend: F%: frequencies of occurrence, A%: relative abundances, D: averagedensities on 100ml of soil.

Nematodesencountered	Densities/100ml	Frequencies %	Abundances %	Types F%
Pratylenchus	97,75	100	14,99	Constant
Tylenchus	138,25	100	21,20	Constant
Tylenchorhynchus	22,75	75	3,49	Constant
Scutellonema	80,75	100	12,38	Constant
Meloidogyne	93	100	14,26	Constant
Helicotylenchus	219,5	100	33,67	Constant

Six genera of plant-parasiticnematodeswereidentifiedacross all plots. Theyweredividedinto five families of the orderTylenchida. The frequency of occurrence and relative abundance made it possible to analyze the structure of the populations. The resultsobtainedshowedthat the 6 generaobserved are all constant with a frequency of occurrence greaterthan 50% (Table 3).

Nematodedensityaccording to soil texture

The faunisticanalysisshowedthat the highestaverage population densitywasrecorded in the 3rd plot with an averagedensity of 207 individuals / 100ml of soil), and itis in this plot that the sand and availablephosphorus rates are higher, respectively 69% sand and 23.38 ppm (Table 1). Hence the distribution of phytoparasiticnematodescouldbepartlylinked to porosity, but also the good health of the host plants. Indeed, the granular structures characteristic of sandysoilspromote the movement of juveniles. Nematodes move inside the pores of the soil, their migration to the rootswillthereforedepend on the type of structure.

Discussion:-

This studyallowed us to identify 6 genera of phytonematodesbelonging to five families in the prospectedfields. The generaencountered are: Tylenchus, Helicotylenchus, Meloidogyne, Pratylenchus, Scutellonema and Tylenchorhynchus. Other information wasprovided on the diversity, abundance and frequency of nematodesassociatedwithcowpea in the marketgardening area of Sotuba. Of the 6 generaencountered, Helicotylenchus and Tylenchus are the densestwith 55% of the nematodesidentified. To ourknowledge, there has not been muchresearch on parasiticnematodesassociatedwithcowpea. However, the importance of the genusHelicotylenchusfoundduringthisstudyconfirms the results of [16]who report thatPratylenchus, Tylenchorhynchus and Helicotylenchusrepresent 98% of the nematodesidentifiedduringtheirstudy in Burkina Faso. Otherstudies have revealed the presence of severalspecies of parasiticnematodes of cowpeathatreduceyield by causingeconomicallysignificantlosses. Thus, research on nematodesassociatedwithcowpeawascarried out in the Saria Agricultural Research Station. This studyshowedresultswith a greaterdiversity of up to 7 species[10]. Anotherstudy in Burkina Faso took place in differentagroecological zones, with a higherspecificdiversity of 12 genera[17]. An almostsimilarresult in terms of diversitywasfound in Bamako on marketgardencropsincludingcowpeawith an averagedensity of 830 nematodes / dm^3 of soil (Larwanou, 2023). Regarding the influence of soilfactors, ourresults are consistent withthosefound in the peri-urban area of Bamako by [12]who report that the porosity of sandy-texturedsoils and the good health of plants provided by nitrogen and phosphorus are factors favorable to the development of phytonematodes.

Conclusion:-

In thisstudy, sevengenera of nematodeswereidentified in the cowpearhizosphere. The generaHelicotylenchusand Tylenchus are the most important in terms of abundancewith more than 50% of the nematodesrecorded. Theseidentifiednematodesshoweddifferentgenericdensitieswith a frequency of occurrence greaterthan 50%. The soilanalysisshowed an acidictendency of the soilwith a sandy loam texture. Theseresultscorroboratewith the hypothesisthat in the presence of a host plant, the soil structure influences the composition of nematode populations. The highestproliferation of nematodes 207 individuals/100ml of soilwasnoted in the plot with the highest rate of sand (69%). Such a type of soilisconducive to the development of nematodes in the presence of a host plant.

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