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Interaction between the root-knot nematode, *Meloidogyne incognita* and the damping-off agent, *Pythium aphanidermatum* on African eggplant under shelter

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Abstract

A sheltered interaction study was conducted to evaluate the effect of the simultaneous presence of the *Meloidogyne incognita* gall nematode and *Pythium aphanidermatum* on the African eggplant variety UVPP. The work was undertaken on a completely random device. The work consisted in comparing the effect of combined treatment (*Pythium* + *Meloidogyne*), treatments with *Pythium* alone and *Meloidogyne alone* to an absolute control treatment without nematode or fungus. The damage caused by the pathogens taken individually is lower than those generated by the parasite complex. Simultaneous inoculation of *Meloidogyne* and *P aphanidermatum* revealed an antagonistic effect on plant mortality caused by the fungus and the severity of root galls caused by nematodes. 67% of plant mortality was noted in *Pythium* alone treatment compared to 33% for *Pythium* + *Meloidogyne* treatment.

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Introduction

African eggplant (*Solanum aethiopicum*) is a very important vegetable crop in sub-Saharan Africa. It is one of the most commonly consumed fruit vegetables in tropical Africa (Fondio *et al*., 2008). It is cultivated in all seasons almost everywhere in Mali except in the northern regions in market gardens. World production is estimated at nearly 50 million tonnes per year. In monetary terms, it reaches a value of 10 billion US Dollars per year (Taher *et al*., 2017). These figures make eggplant the fifth most important nightshade after potatoes, tomatoes, peppers and tobacco (FAO, 2014). China, India, Egypt, Turkey and Iran are the biggest producers. Their productions vary from 28 million tonnes for the former to 75,000 tonnes for Iran. In Africa, an estimate in a few countries gives an annual production of 99000 tonnes in Côte d'Ivoire, 8886 tonnes in Niger and 4729 tonnes in Senegal (FAO, 2019). In Mali, *S. aethiopicum* ranks third in fruit vegetable production with 16% after okra 42% and tomato 29% (Ministry of Agriculture, 2018). Immature fruits are part of the daily diet of the Malian people, but in East Africa in addition to fruits, leaves and young shoots are also eaten fresh or used in the sauce.

Despite this significant production, very little research has been done on this crop. Thus most of the cultivated varieties are local and are subject to severe attacks from insects and diseases such as damping off, undiagnosed leaf diseases, or wilting of the plants. These wilts can be bacterial or caused by fungi such as *Fusarium spp*. and *Pythium spp*. This situation is observed in the market gardening sites of Bamako where strong attacks of *Pythium spp*. were regularly recorded during the rainy season. Other pathogens such as root-knot nematodes (*Meloidogyne spp*.) are also very common on African eggplant. They constitute one of the main constraints to the production of vegetable crops in general. All the market garden sites visited between Kati (Koulikoro region) and the Niger Office (Ségou region) were heavily infested. But these nematodes are rarely isolated alone from the withered plants analyzed. They are generally associated with other soil-borne pathogens (fungi or bacteria) which contribute to aggravate the damage. Nematodes interact with many fungal and bacterial diseases (Kilalo, 2019). Several types of interactions between nematodes and other pathogens have been reported. These interactions can be synergistic when the damage of the pathogen complex is greater than the sum of the individual damage of the two pathogens 1 + 1> 2. Conversely, they are said to be antagonistic when the damage of the complex is less than the sum of the individual damage 1 + 1 <2 (Back *et al*., 2002). In Mali, the interaction between phytonematodes and fungi has never been studied. The objective of this study is therefore to determine the type of interaction between nematodes of the genus *Meloidogyne* and *Pythium aphanidermatum* on African eggplant.

Material and methods

The plant material used was composed of two cultivars: tomato (*Solanum esculentum*) cv Roma VF for the breeding of *Meloidogyne* spp and African eggplant (*Solanum aethiopicum*), variety UVPP for the study of the interaction between nematodes and *Pythium spp*. The seeds were provided by the World Vegetable Center (AVRDC) seed bank. The nematodes and fungus originate from plants infested with okra, tomato, eggplant, chili, roselle, amaranth, bean, chorchorus, and onion. These plants were collected in market garden sites around Bamako.

Nematological parameters

Extraction of egg masses

This operation was done under a stereoscope. Only roots showing galls in good condition were used. The roots were first washed under a thin stream of tap water to remove soil particles and saprophagous nematodes. They were then disinfected with 1% active chlorine bleach for one minute, then rinsed with water. After they were placed in a petri dish using a scalpel and a lance-shaped needle, the gall was opened superficially.

The egg mass in a mucus placed on the posterior end of the female under the bark was torn off and then placed in a beaker containing 25 ml of distilled water

for hatching. Two days later, the resulting larvae were inoculated into young tomato plants.

Nematode breeding

The breeding was done in plastic pots filled with sterilized soil. A cv Roma tomato plant was transplanted into each pot. The plants were inoculated with the second stage juveniles (D2) by pouring a suspension of 300 juveniles into a small hole dug near the crown of each plant. This breeding lasted a month.

Extraction of root nematodes

The nematodes were extracted using the Maceration-Filtration method (Coyne *et al*., 2007). This method involves washing the roots under running tap water. They are then cut into small pieces with pruning shears and disinfected by soaking in a bleach solution for 4 minutes to thin them out. The roots are then rinsed with tap water for 15 minutes and macerated in a kitchen blender for 30 seconds.

The macerate is then passed through a series of three sieves: 150 µm, 75 µm and 38 µm inclined at 45 degrees to maximize the harvest. The filtrate collected by the last sieve contains larvae and nematode eggs. To have more inoculum, the macerated root residues were placed for two days on small sieves reinforced with Kleenex, the whole placed in a plastic plate containing water. The captured eggs and larvae were used to inoculate young tomato plants cv Roma VF.

Identification of the Meloidogyne species

Fixation of nematodes

The roots were thinned with bleach for 4 minutes and then rinsed with tap water for 15 minutes to remove the bleach residue. They were then boiled for 30 seconds in 30 ml of distilled water plus 1 ml of a stock solution of fuchsin acid (0.35 g fuchsin acid; 25 ml acetic acid; 75 ml distilled water) and then cooled for 30 minutes at room temperature.

They were decolorized in acidified glycerol solution by adding 6 drops of nitric acid before being boiled. The discolored roots were freed from glycerol and then placed in a petri dish containing lactophenol for observation of females and egg masses. A root segment observed under a microscope shows red-stained nematodes and discolored root tissue. This facilitates the dissection of females and the preparation of the perineal plates.

Preparation of the perineal plates

Dissection of the attached female nematodes was performed under a stereoscope on a microscope slide. The females thus fixed with fuchsin acid were fished and placed on a slide object in a drop of distilled water, then using the spines of an entomologist the female was pierced and emptied of its contents by pressing lightly on the nematode. They were then cut in the anterior part to preserve the perineal part. For complete cleaning of the preparation, the excess water was sucked up with a blotting paper. A drop of glycerol or lactophenol was then added to the preparation which is ready for microscopic observation of the perineal plaques. These perineal figures were compared to those published by Eisenback (1985).

Fungal parameters

Cultivation of Pythium spp

Fragments of infested plants were disinfected with 1% active chlorine bleach for 1 minute, then rinsed three times with distilled water and dried. These fragments were then placed on Cornmeal agar (CMA) composed of 60 g of corn, 15 g of amended Benomyl agar 100 ppm; PCNB 100 ppm and ampicillin 60 ppm (Plaats-Nitterink, 1981).

All operations were performed under a horizontal laminar flow hood. After three days, explants taken from the end of the mycelium were placed in new petri dishes containing CMA. Two weeks later, the cultures were dried in a laminar flow hood with the lid open slightly. Drying stimulates the germination of oospores (Ruben *et al*., 1980.). The contents of two dried petri dishes were crushed at a time for 5 minutes in 100 ml of distilled water using a kitchen blender. The ground material obtained was centrifuged at a speed of 10,000 revolutions per

minute for 10 minutes. Using Pasteur pipettes, the supernatant containing the agar and the mycelial fragments were aspirated, leaving the pellet with a high concentration of oospores in the bottom of the tube. The concentration of oospores was measured using a hemacytometer. The final concentration was adjusted to 20,000 oospores per ml. 10ml were inoculated on the eggplant plants.

Identification of the Pythium species

Identification of *Pythium spp* was made using the key from Plaats-Niterink, (1981). Thirty-two oogonia and sixteen mycelial filaments were used. The following morphological characters were observed: the general organization of the thallus, the position of the antheridia in relation to the oogonium, the shape of the sporangia, the space occupied by the oospore inside the oogonium and the presence or the absence of projection on the oogonium.

Using Pasteur pipettes, the supernatant containing the agar and the mycelial fragments were aspirated, leaving the pellet with a high concentration of oospores in the bottom of the tube. The concentration of oospores was measured using a hemacytometer. The final concentration was adjusted to 20,000 oospores per ml. 10ml were inoculated on the eggplant plants. The formation of sporangia and zoospores was induced by placing fragments of mycelium in a petri dish containing an equal volume mixture of river water and distilled water. This mixture was autoclaved at 120 °C for 20 minutes. A leaf of wild grass (*Pennicetum penicellatum*) previously boiled for 10 min was placed in the petri dish for 3 days at room temperature (Olson *et al.*, 2016). The size of oospores, oogonia and the width of hyphae were measured. The dimensions found were compared with those described by Plaats-Nitterink (1981).

To verify Koch's postulates, *P. aphanidermatum* was re-isolated in pure culture on Cornmeal agar from withered plants. Plants not wilted but inoculated with *Pythium* were also examined. Fragments of the stems were washed with sterile distilled water before use.

Study of the nematode and fungus interaction

The eggplant seeds were disinfected by soaking for three minutes in a solution of sodium hypochlorite at 1% active chlorine, rinsed twice with sterile distilled water and dried, treated with Apron star: a fungicide and insecticide, then dried. The transplant pots were washed thoroughly with soap before filling them with pasteurized soil. Sowing was carried out in wooden planks filled with previously pasteurized potting soil. The substrate used was formed by a mixture of earth (three volumes), fluvial sand (one volume); rice husk (5kg); fertilizers (5kg of compost, 120g of NPK) and an adequate quantity of water. Three weeks after emergence, the young eggplant plants were transplanted into pots. 48 hours after transplanting, nematodes and fungi were inoculated therein.

The tests were carried out in 10 cm^3 pots filled with pasteurized soil. Four treatments and twelve pots/treatment and one plant per pot were used. The treatments are as follows:

To (without nematodes, without *Pythium*, 10ml of water)

T1: *Meloidogyne* alone (300 J2 / plant / pot)
T2: *Pythium* alone (10 ml of oospore suspension, 2.105 oospores/plant / pot)
T3: *Meloidogyne* (300 J2) + *Pythium* (2.105 oospores / plant / pot).

The treatments were applied one week after transplanting the eggplant plants. A completely random device was used. Follow-up of the interaction lasted 30 days after inoculation. The rate of wilt caused by *Pythium* was evaluated. Withered plants were uprooted and the parasite isolated in pure culture on Cornmeal agar for confirmation.

The dry weight of the collar cut plants was determined. The plants were then stripped and the roots washed thoroughly under running tap water to assess the root gall index. This root gall index was estimated using a scale of 0 - 5: 0 = no gall, 1 = traces of infection with a few small galls, 2 = more than 25%

of the roots have galls, 3 = from 25 to 50% of roots bear galls, 4 = 50 to 75% of roots bear galls; 5 = more than 75% of the roots carry galls (Fayzia *et al*., 2018).

Statistical analyzes

The data for the gall index were transformed into severity by dividing each index by the highest possible score and multiplying the whole by 100. The raw data for the different characteristics were collected and subjected to an analysis of variance (ANOVA) at P = 5% (Table 1).

Results and discussion

Meloidogyne identified

All the plates observed present a more or less square high dorsal arch with lateral fields marked by breaks between the dorsal and ventral cutmarks (Fig. 1, D). These striations are generally smooth to wavy at the level of the lateral fields. These characteristics confirm that it is *Meloidogyne incognita*.

The Pythium identified

All isolates and cultures yielded only one species of *P aphanidermatum*. On Cornmeal agar, the *P aphanidermatum* produces an abundant mycelial felting of whitish color (Figure. 2). The mycelial filaments are coenocytic and the diameter of the hyphae is between 2.5µm and 7µm. The oogonia are spherical and smooth without projections. The oospores are aplerotic and the antheridium sac-like. The sporangia are in the form of elongated vesicles sometimes swollen.

Table 1. Effects of *Meloidogyne* spp and *P. aphanidermatum* alone or in combination on eggplant wilt, root severity and plant dry weight. NS = not significant; S = Significant.

Treatments	Dry weight (g) NS	Severity (%) S	wilt (%)
Nematode alone	6,99	60	0
Nematodes + *Pythium*	8,98	38	33
Pythium alone	4,5	0	67
Witnesses	7,86	0	0
Values	0,68	0,42	

According to Koch's postulates, the strain was re-isolated from withered plants in pure culture on Cornmeal agar and tentatively identified as *P aphanidermatum* based on morphological characteristics. This result demonstrates that the pathogen colonized the roots of the plant.

These descriptions are consistent with those of *P. aphanidermatum* (Figure. 2) reported by Ashwathi *et al* (2017). The differences in the diameters of the oospores described here can be justified by the temperature differences of 29 to 30°C under which the *aphanidermatum* was cultivated instead of 25°C. *P. aphanidermatum* is adapted to high temperatures where very few species can grow.

This adaptation may explain the predominance of this species in Mali. This species has been isolated from okra, tomato, chili, eggplant and roselle. On these crops, it causes extensive damage. It causes the same type of symptoms everywhere: damping-off, rotting and blackening of the crown and sometimes of the stem. In conditions of high humidity, all aerial parts and fruits can be colonized.

In Tanzania *P aphanidermatum* and other soil microorganisms such as *Fusarium* spp. and *Rhizoctonia* spp are major constraints to bean production (Binagwa *et al*., 2016). These pathogens act individually or as parasitic complexes (Aghale *et al*., 2017).

Effect of interaction

Death of the plants occurred one week after inoculation with *P aphanidermatum*. Of the 12 plants inoculated, 8 had died in the *Pythium* treatment alone, which represents 67%. In the *Pythium* + *Meloidogyne* treatment 4 plants out of 12 were dead,

ie 33%. Inoculation with *M. incognita* alone caused only one plant death, or about 8% of the plants in the treatment. The mortality caused is twice as high in the treatment of *Pythium* alone (67%) than in that *Pythium* + *Meloidogyne* (33%). In the control treatment, there were no dead plants.

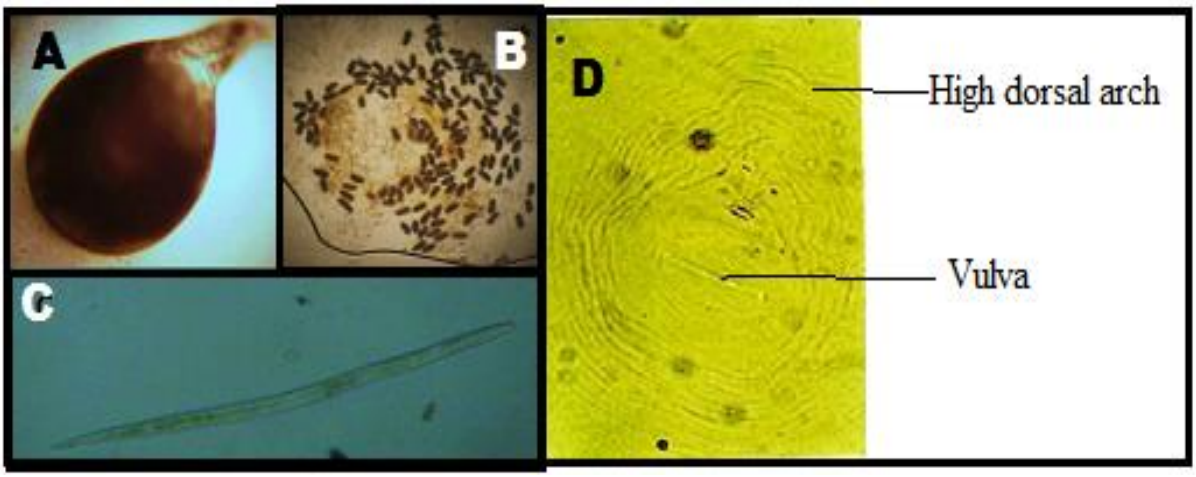

Fig. 1. Root-knot nematodes associated with vegetable crops, some stages of development: A = female *Meloidogyne*, B = egg mass, C = second-stage larva, D = perineal plate of *M. incognita*.

Unilateral Student Test of the severity of the attack on the roots shows a statistically significant difference (P <5%) between treatment with *M. incognita* alone and the combination of the two organisms. The mean severity of galls is 60% for *M. incognita* alone and 0% for *Pythium* alone, against 38% for *P. aphanidermatum* + *M. incognita* (Table 1), ie a difference of 22%. The results of plant wilt rate and gall severity support the hypothesis of an antagonistic interaction between *Meloidogyne* and *P aphanidermatum*. In both cases, the damage caused by the sum of the pathogens individually is lower than That generated by the parasite complex (1 + 1 <2). Pythium treatment alone gave the lowest dry weight 4.5g while the combination *Meloidogyne* and *P aphanidermatum* gave a dry weight of **8,** 98g.

The nematode treatment alone and the control gave dry weights of 6.99g and 7.86g respectively (Table 1).

Statistical analysis of the dry weight data showed that there was no significant difference between the results (p> 5%). The dry weights of withered plants have not been determined. These results confirm an antagonistic trend in the effect between *Meloidogyne spp* and *P. aphanidermatum*. The *P. aphanidermatum* and *M. incognita* complex visibly reduced plant wilting: 33% deaths in the *Pythium* + *Meloidogyne* treatment against 67% in the *Pythium* treatment alone.

The severity of the galls confirms this trend: 60% in the *Meloidogyne* treatment alone, against 38% for the *Pythium* + *Meloidogyne* complex. This result is explained by the fact that the plants were simultaneously inoculated with both organisms. *Pythiums* are necrotrophic pathogens that feed on the dead cells of host plants after killing them.

On the other hand, *Meloidogyne* spp are obligate parasites. So with the death of root tissues caused by *Pythium*, the nematodes could no longer develop.

Pathogen interactions have been reported by several authors and their research results vary from one complex to another. Thus, a study in the United States of America has shown that extracts of *Aspergillus candidus* reduce mobility or even completely destroy second instar larvae of M. incognita (Shemshura *et al.*, 2016. In India, an interaction study was carried out under greenhouse conditions. The effect of the complex is increasing the suppression of the growth of tomato plants (Kumar *et al.*, 2017).

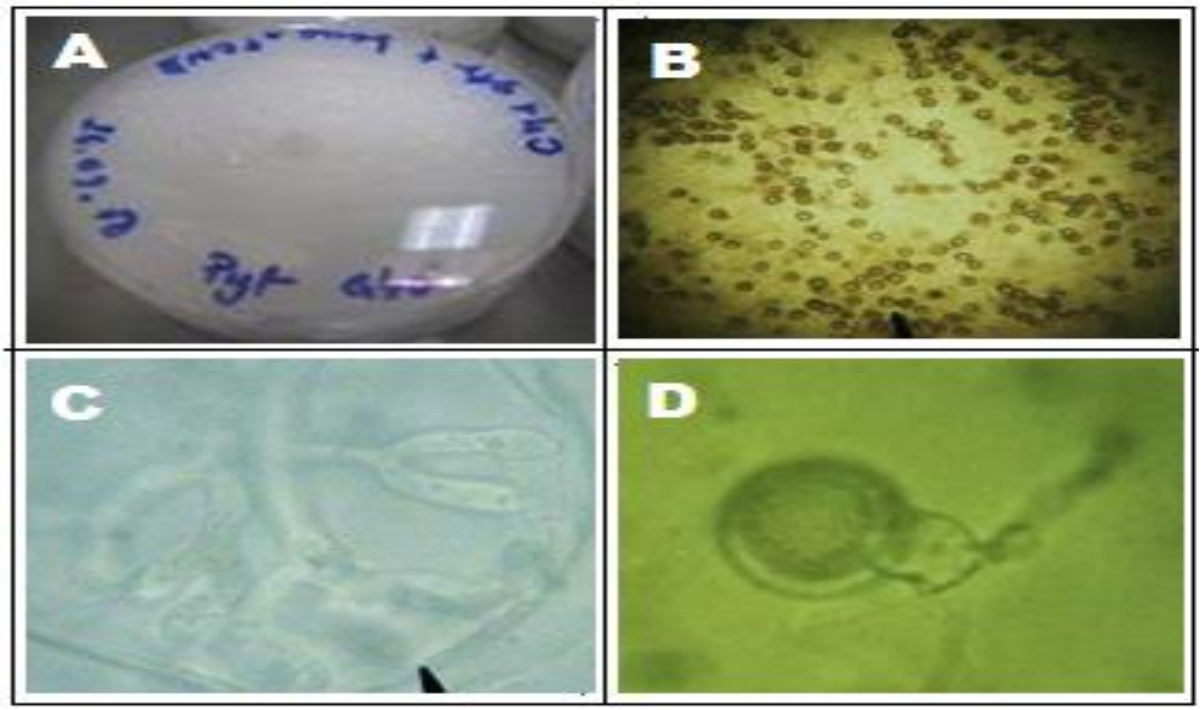

Fig. 2. Some characteristic organs of *P. aphanidermatum*: A = pure culture; B = oospores; C = branched sporangia; D = Oogonium and antheridium attached.

This antagonistic interaction found between *M. incognita* and *P aphanidermatum* is consistent with that obtained in Canada where it has been shown that the presence of soybean cyst nematode allows *Fusarium virguliforme*, the causative agent of sudden death syndrome, to have more impact on soybean mortality (Carolane, 2019).

Conclusion

From this study, an antagonistic type interaction emerges. The parasite complex contributed to the reduction in wilting of the plants compared to the *Pythium* treatment alone. The same is true for the severity of root galls, which is greater in the treatment of *Meloidogyne* alone than with the complex.

This result may be due to the fact that the plants were concomitantly inoculated with both organisms. Since *Pythium* is necrotrophic, it feeds on the dead cells of the host plant after killing it. On the other hand, *Meloidogyne* is an obligatory parasite. So with the death of root tissues caused by *Pythium*, the nematodes could no longer develop. Further research should be directed towards stepwise inoculation of pathogens.

References

Back MA, Haydock PPJ, Jenkinson P. 2002. Disease complexes involving plant parasitic nematodes and soilborne pathogens *Plant Pathology* **51,** 683-697. Review Article Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK 683-697.

Barker KR, Carter CC, Sasser JN. 1985. In: An advanced Treatise on *Meloidogyne*. **2,** Methodology. Raleigh, North Carolina State University Graphics. 223.

Binagwa PH, Conrad KB, Susan NM, Inocent IR. 2016. Morphological and molecular identification of *Pythium spp*. isolated from common beans (*Phaseolus vulgaris*) infected with root rot disease Journal of Plant Science **10(1),** p 1-9, January 2016. http://dx.doi.org/10.5897/AJPS2015.1359. http://www.academicjournals.Org/*African*

Bouriquet G, Delassus M. 1963. Présence au Mali sur le tabac de *Pythium aphanidermatum*. L'Agronomie Tropicale. Série 3, Agronomie Générale. Etudes Scientifiques **18(3),** 323-327.

Carolane A. 2019. Interaction entre le nématode à kyste (*Heterodera glycines*) et *Phytophthora sojae* chez le soya. Mémoire de Maîtrise en biologie végétale. Université Laval Québec, Canada. 55 p. Collection: Origin, Composition, Seed Dissemination and Utilization in Breeding. Front. Plant Sciences **8,** 1484.
http://dx.doi.org/10.3389/fpls.2017.01484.

Coyne DL, Nicol JM, Claudius-Cole B. 2007. Pratical plant nematology: a Field and laboratory guide. SP-IPM. Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin. 82 p.

FAO. 2014. Production Databases. Available online at:
http://www. faostat.fao.org (Accessed January 30, 2017).

FAO. 2019. FAOSTAT Production Databases available online at:
http://www. Faostat.fao.org. consulté le 20 juillet 2019.

Fayzia MM, Amira SS, Khalil AEM, Shawky SM, Taher AA. 2018. Seasonal fluctuation and biological control of root knot nematodes *Meloidogyne incognita* on cucumber. Pakistan Journal of Nematology. ISSN 2313-1942, **36(1),** 71-81.

Fondio I, N'Tamon IN, Hala FN, Djidji HA. 2008. Evaluation agronomique de six cultivars d'aubergine africaine (*solanum spp*.) de la nouvelle collection des plantes légumières du CNRA agronomie africaine **20(1),** 69–79.

Kilalo D. 2019. Transmission et propagation des maladies et des arthropodes nuisibles Département de Phytologie et Protection des Cultures 134 p.

Kumar N, Jayant B, Ratan LS. 2017. Interaction between Meloidogyne incognita with Fusarium oxysporum f. sp. lycpersici on Tomato. International Journal of Current Microbiology and Applied Sciences **6(8),** 1770-1776.
https://doi.org/10.20546/ijcmas.2017.

Ministère de l'Agriculture du Mali. 2018. Statistiques de la Direction Nationale de l'Agriculture sur la production des cultures maraîchères. Aout **09,** 2018.

Olson JD, Damicone JP, Kahn BA. 2016. Identification and charactérisation of isolates of Pythium and Phytophthora spp. From snap beans with cottony leak. Plant Diseases **100,** 1446-1453.
http://dx.doi.org/10.1094/PDIS-06-15-0662-RE.

Ruben DM, Frank ZR, Chet I. 1980. Factors affecting Behavior and Developmental Synchrony of Germinating oospores of *Pythium aphanidermatum*. Agricultural Research Organisation, Bet Dagan, Israel. Pathology **70,** 54-59.

Rusuku G, Buruchara RA, Gatabazi M, Pastor-Corrales MA. 1997. Occurrence and distribution in Rwanda of soilborne fungi pathogenic to the common bean. Plant Diseases **81,** 445- 449.

Shemshura ON, Nadiya EB, Mariya NM, Susan LFM, Clifford PR, Edward PM. 2016. Isolation and identification of nematode-antagonistic compounds from the fungus *Aspergillus candidus*. FEMS Microbiology Letters **363(5),** p 9.
http://dx.doi.org/10.1093/femsle/fnw026

Taher D, Solberg S, Prohens J, Chou Y, Rakha M, Wu T. 2017. World Vegetable Center Eggplant Collection: Origin, Composition, Seed Dissemination and Utilization in Breeding. Frontiers in Plant Science REVIEW published.
http://dx.doi.org/10.3389/fpls.2017.01484. 12

Touré BK. 2017. Les nématodes des cultures maraîchères: Dynamique saisonnière des peuplements et détermination des espèces de *Meloidogyne* dans la zone péri-urbaine de Bamako. Thèse de Doctorat. Université des Sciences des

Techniques et des Technologies de Bamako. Faculté des Sciences et des Techniques, p 170.

Van der Plaats-Niterink J. 1981. Monograph of the genus Pythium. Studies in Mycology 21. Baarn, The Netherlands: Centraalbureau voor Schimmelcultures, 25-39.