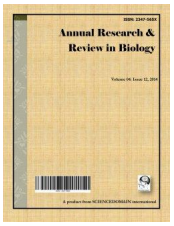

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Fungal Communities in Roots, Soil Inhabiting Nematodes and Physico-chemical Parameters of Soils in Three Farms of Commercial Strawberry Production in Moulay Bousselham (Morocco)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to determine fungal communities associated to strawberry plant roots, densities of nematodes in fumigated soil and physico-chemical properties of soil in three commercial strawberry plots (Dlalha, Ouled Aguile and Gnafda) located in Gharb region and visited three times during 2013-2014 season. Under a slight variation of soil temperature and humidity with specific physico-chemical characteristics, a high infestation level of soil by nematodes was marked in the studied farms over the periods of sampling. The lowest number was observed in the first visit attaining 1000 units/g of soil, increased to 5000 units/g of soil in the second sampling period at one site and in the last visit, a significant decrease trend occurs for the big plot which the number of nematodes was reduced to 1500 units/g of soil. The analysis of fungal communities colonizing roots of strawberry plants showed the presence of 13 fungal species. *Rhizoctonia solani* presented

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a higher frequencies of isolation from roots of Festival variety reaching respectively 36% and 26.72% in the first and the second visits of Dlalha farms compared to those recorded in the second visit of Ouled Aguile (18%) and Gnafda (13.6%) farms. The *Fusarium* genus was represented by *F. solani, Fusarium* sp.1 and *Fusarium* sp.2 detected at frequencies not exceeding 7.08%. Those of *C. gloeosporioides* reached 12%, *Phytophthora* sp. (15.8%). In addition, the appearance of *A. alternata*, *C. herbarum*, *Aspergillus* sp., *Circinella* sp., *Ulocladium* sp. and *Trichoderma asperellum* was more frequent during the three sampling periods.

Keywords: Strawberry; nematodes; soil temperature; soil humidity; fungi distribution; root colonization; fumigated soil; Morocco.

1. INTRODUCTION

Strawberry (*Fragaria ananassa* Duch.) is one of the most economically important crops worldwide [1,2,3]. It is grown under a wide range of climatic conditions as wild and cultivated plants producing small delicious fruits [4]. However, the strawberry cultivation is constrained by serious diseases involving different soil borne pathogens which severely impacts the plant agronomic performance and generates economic losses in conventional production fields [5] and are problematic especially when they remain alive in soil under unfavourable conditions for many years [6]. Many species have been reported to cause strawberry root rots, crown rot and damping off diseases in several countries where strawberry cultivation is more extent [7,8,9,10]. Their causative agents include *Fusarium oxysporum* [11,12], *Macrophomina phaseolina* [13,14], *Pythium* spp. [15,16,17], *Phytophthora* spp. [18] and *Rhizoctonia* spp. [19]. In addition, the cultivated strawberry can be parasitized by plant parasitic nematodes, which are known to cause a reduction in strawberry yield [20,21]. There are two most common species known as root- knot nematodes [22] and root-lesion nematodes [23,24,25]. These organisms are regarded as a primary cause of black root diseases [22], as predisposing factor for strawberries infection by Rhizoctonia and Pythium [15] and enhancing damping-off diseases caused by various fungi [20,26]. But, environmental factors and cultural practices also play an important role in the probability of disease outbreaks and development [27,28,29]. Indeed, the virulence and the dominance of some pathogens are influenced by the prevailing seasonal temperature regime [30]. Environmental stresses including soil compaction, excess moisture, and winter injury, also may increase the severity of diseases [31,32]. Previous studies have reported environmental effects like soil temperature and soil water on nematode population trends [33]. In Morocco, surveys conducted from 2010-2013 have identified mycoflora associated to strawberry plants grown in the major berry-producing areas of Gharb-Loukkos region which the distribution was variable in the prospected farms [34,11,17]. However, information on the distribution, the occurrence of plant-parasitic nematodes, soil-borne fungal species associated with strawberry and changes in abiotic variables during growing season is non-existent. Thus, the main objective of the present study is to follow the appearance of telluric fungi colonizing bellow ground parts of strawberry plants (root-associated fungi), nematodes inhabiting the rhizosphere around the roots of strawberry plants and soil parameters varying during growing season.

2. MATERIALS AND METHODS

2.1 Study Site and Location

The surveys were conducted during the crop season of 2013-2014 (which starts mi- August and ends June) at the strawberry growing farms covering three villages belonging to the town of Moulay Bousselham in the south of Loukkos and delimiting the northern Atlantic coast of the Gharb region (70 km north of Kenitra and 35 km south of Larache). Three selected plots G, M and P, were visited three times: in February 04, 2014 (S1), in March 03, 2014 (S2) and in April 24, 2014 (S3) (Table 1).

2.2 Soil and Strawberry Plants Sampling

To monitor nematode numbers, soil samples were taken three times during the strawberry growing seasons (full growth and flowering stages). At each visit during the strawberry growing seasons, 5 sites for each farm were

Table 1. The sampling sites surveyed in the municipality of Moulay Bousselham during the 2013-2014 strawberry campaign

Localities	Farmers	Farm size	Age and type of culture	Soil disinfestation	Varieties
Dlalha	Allal (G) Abd lkbir (M) Hassan (P)	Big size (28 Ha)	Second year Greenhouse	Fumigation metam sodium 2012-2013	Festival
Ouled Aguile	Abd lkbir (M)	Medium size (1.5 Ha)	Second year under tunnels	Fumigation with metam sodium 2012-2013	Festival
Gnafda	Hassan (P)	Small size (0.6 Ha)	First year under tunnels	Fumigation with metam sodm 2012-2013	Camarosa

randomly selected. A sample of one plant with soil attached to the roots was taken from each site, carefully placed inside a bag for transfer to the laboratory. Upon arrival soil adhering to the roots of 5 strawberry plants was scraped into the same bag, mixed to yield a composite soil sample and the plant removed. The remaining soil in the bag was thoroughly mixed before 100 g was removed for placing in a labelled plastic bag for nematode extracting.

2.3 Isolation of Fungi from Strawberry Plants

Isolation was done from thinner roots, cut into small segments of 1cm length from adjoining areas of diseased and healthy areas of the strawberry plants. Root pieces were washed under tap water for about 30 minutes to remove any dirt or soil particle. The root pieces were disinfected in sodium hypochloride solution at a concentration of 5% then with 95° alcohol for about 2 minutes and then passed from two washes of distilled sterile water for 2-3 minutes each. The treated root pieces were dried completely and then transferred to Petri dishes containing sterilized potato-saccharose agar medium (200 g of potato starch, 15 g of sucrose, 20 g of Agar-agar, 1000 mL distilled water) supplemented with 5 mg streptomycin. All the plates were kept at 25 ± 1°C for 5 days. The fresh growth of the fungi was transferred to freshly prepared potato-dextrose agar medium for sub-culturing under the same conditions for 7 days. The growth was sub-cultured/multiplied whenever needed during the entire study. The fungi isolated were identified by studying their typical mycelial growth produced on the potato dextrose agar medium and conidial morphology using standard diagnostic keys of Tarr [35], Ellis [36], Chidambaram et al. [37], Domsch et al. [38], Champion [39], Ponchet [40].

The percentage of infection and/or contamination by different fungal species was calculated using the method of [41] which defines the frequency of isolation of different fungi from 100 lesions root rots present on the plants studied according to the formula:

$$PC = (NFI / NTF) \times 100$$

Where PC represents infection ratio and / or contamination;

NFI is number of lesions infected with a fungal species;

NTF is the total number of lesions.

2.4 Nematode Analysis

Nematodes were extracted by processing 100 g of the homogenized soil/plot. Thus, a modified Baermann method was used [42]. The method involves placing a screen on top of a bowl. Tissue paper is placed on top of the 50 micron mesh screen and a thin layer of the soil sample is placed over it. The bowl was filled with tap water until it covers the soil sample. After a set period of 48 hours the tissue containing the soil and screen is removed and the water in the bowl is collected in a beaker. The nematodes are collected in the water left behind in the beaker. They will then concentrate and sink to the bottom. 25 mL of the filtrate was collected, poured into a gridded Petri dish, and then the number of nematodes is counted under an optical microscope at a magnification × 40. The count is repeated three times. A mean of 3 counts was taken in each case.

$$Nmn = (\Sigma Nb) / (Nr)$$

Nmn: Mean number of nematodes
Nb: Number of nematodes in each repetition;
Nr: Number of repetitions.

The rule of three counts was used to estimate the number of nematodes per 100 g soil.

2.5 Temperature and Relative Moisture

At every visit, a diagonal randomly sampling was conducted per plot, the relative moisture and temperature of soil were measured using portable Thermo-hygrometer, inserted at 15 cm depths on each strip plot.

2.6 Physico-chemical Analysis of Soil

At the first visit, 5 samples of about 5 Kg collected in the rhizosphere of the strawberry plants selected on the diagonal of each plot surveyed, were mixed to yield composite soil samples.

Physical and chemical parameters of soil such as pH, electric conductivity (EC), organic matter (OM), nitric nitrogen (N Nit), ammonia nitrogen (N Amo) mineral nitrogen (N. Min), phosphorous (P) and potassium (K) were determined through conventional analyzes in the Laboratory soil tests of the Regional Office of Agricultural Development GHARB (ORMVAG).

2.7 Statistical Analysis

The data for all measured parameters were subjected to analysis of variance and significant differences between means were evaluated using Least Significant Difference Method at $P<0.05$ (LSD test), a comparison test of means is applied to the data.

3. RESULTS AND DISCUSSION

The field soils displayed variable amounts of total carbon, nitrogen and phosphorus while pH was almost alkaline during the investigation periods. As shown in Table 2, the pH value of the sandy soil are almost the same (basic around 7.90), with a low electric conductivity value varying from 0,09 to 0,15 mmhos/cm, poor contents in organic matter which the percentage reached 0.96% in soil of P and no limestone in M and P farms. The total nitrogen content of the soil samples is variable. For ammonia nitrogen content, is ranged from 19.08 ppm in Ouled Aguile to 24.48 ppm in Dlalha. Highest amounts of mineral nitrogen and nitric nitrogen were marking the soil of Gnafda followed by those of Dlalha. Also, the soil samples contain more potassium, of the order of 205 ppm in M and 176 ppm in G compared to reduced amounts of phosphorous (Table 2).

The measure of soil temperatures during the visits from February to April revealed a slight fluctuation (Fig. 1). In the first period (February), the soil temperature differed significantly and it was ranged between 20°C and 25°C. In the follower month, a slight increase was noticed but there were no differences between the three sites where soil temperatures were around 30°C. In the third period, it was significantly equal to that recorded in the previous period although the difference between GS3 and PS3 or MS3 (Fig. 2).

As for soil moisture, it registered a high level between 50 and 60% in the first visit for all of plots, decreased below 50% in the second visit then went up to more than 50% in the last visit (Fig. 2).

The total number of plant parasites nematodes found in the soil samples revealed a high infestation level varying among the studied farms and period of sampling. The lowest numbers were observed in the first visit attaining 1000 units/g of soil (Fig. 3). A significant difference of nematode number was noticed among the three farms in the second visit in which the total number increased reaching almost 5000 units/g

Table 2. Soil properties of the research field in the visited sites

Soil	pH	EC	Limestone	O.M	O.C	Nitrogen (ppm)			P	K
		mmhs/cm	%	%	%	Amo.	Nit.	Min.	ppm	ppm
G	7.96	0.15	0.12	0.95	0.55	24.48	133.92	158.40	31	176
M	7.79	0.11	0.00	0.91	0.53	19.08	126.48	145.56	21	205
P	7.98	0.09	0.00	0.96	0.56	21.24	190.96	212.20	25	88

G, Allal's farm; M: Abd Ikbir's farm; P: Hassan's farm
EC: electric conductivity, OM: organic matter, N Nit: nitric nitrogen, N. Amo: ammonia nitrogen, N. Min: Mineral nitrogen, P: Phosphorous and K: Potassium

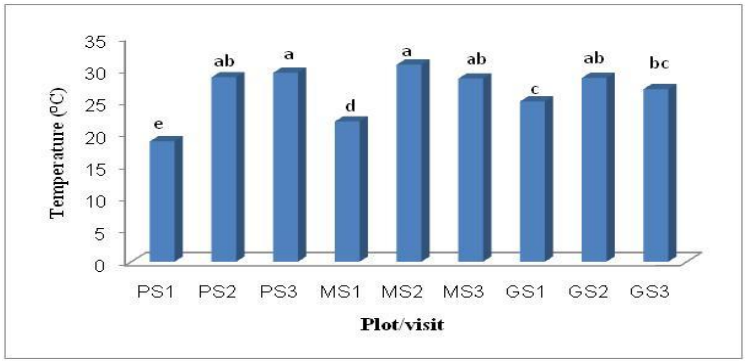

Fig. 1. Soil temperature values of sites in three farms during sampling visits

PS1, PS2, PS3: The three measures separately realized in February (PS1), March (PS2) and April (PS3) at Gnafda parcel.
MS1, MS2, MS3: The measures realized in February (MS1), March (MS2) and April (MS3) in Dlalha parcel.
GS1, GS2, GS3: The three measures realized in February (GS1), March (GS2) and April (GS3) at Ouled Aguile parcel.
Bars with the same letter show no significant difference at 5% level of probability by LSD test

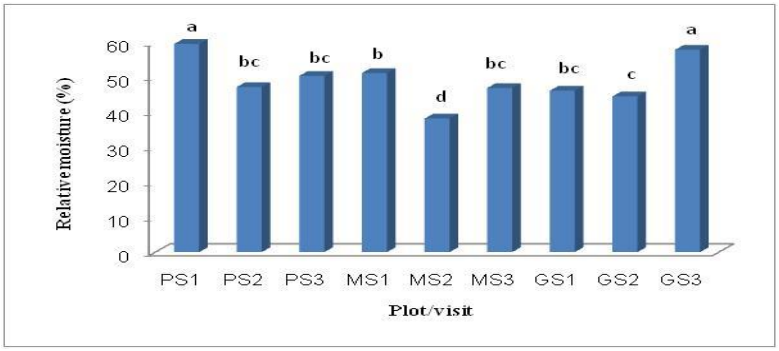

Fig. 2. Relative soil moisture percentages of sites in the 3 farms during each visit times

PS1, PS2, PS2: The three measures separately realized in February (PS1), March (PS2) and April (PS3) at Gnafda parcel.
MS1, MS2, MS3: The measures realized in February (MS1), March (MS2) and April (MS3) in Dlalha parcel.
GS1, GS2, GS3: The three measures realized in February (GS1), March (GS2) and April (GS3) at Ouled Aguile parcel.
Bars with the same letter show no significant difference at 5% level of probability by LSD test

of soil in comparison with those counted in both other farms. In these ones, the nematode number is significantly similar going over 2000 unites/g of soil. While in the last visit, a significant decrease trend occurs since for the big plot the number of nematode was reduced to roughly 1500 units/g of soil. In contrary, Ouled Aguile and Gnafda farms maintain the same level of infestation as in second visit (Fig. 3).

Results from analyses of species colonizing roots of strawberry plants grown in Dlalha, Ouled Aguile and Gnafda farms showed that there were 13 species of harmful fungi (Table 3), among them *Rhizoctonia solani* was predominant. Its isolation frequencies was higher from roots of Festival variety reached respectively 36 and 26.72% in the first and the second visits of Dlalha farms compared to those recorded in the second visit of Ouled Aguile (18%) and Gnafda (13.6%) farms. In contrary, this species was absent in the third prospection of both farms Dlalha and Ouled Aguile. A lower occurrence marked *Colletotrichum gleoesporioides* at the second visit in Gnafda (3.91%) and Ouled Aguile (3.96%) where it showed a frequency superior in the first prospection (12%) but overall null in Dlalha. The Fusarium genus was represented by *F. solani* that appear only in Ouled Aguile and Dlalha at the first visit as well as *Fusarium* sp.1 and

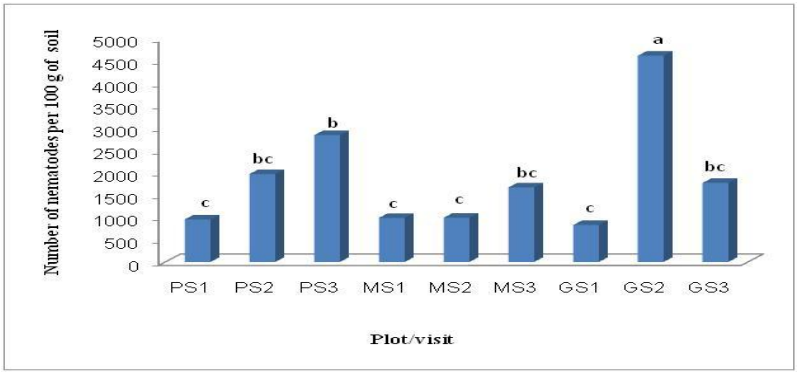

Fig. 3. Number of nematodes recovered from soil samples taken from three sites during three investigation periods

PS1, PS2, PS3: The three measures separately realized in February (PS1), March (PS2) and April (PS3) at Gnafda parcel.

MS1, MS2, MS3: The measures realized in February (MS1), March (MS2) and April(MS3) in Dlalha parcel

GS1, GS2, GS3: The three measures realized in February (GS1), March (GS2) and April (GS3) at Ouled Aguile parcel.

Bars with the same letter show no significant difference at 5% level of probability by LSD test

Fusarium sp.2 showing a lower frequency of isolation except those recorded by *Fusarium* sp.1 in Dlalha at the last visit (7.06%) and 3.08% by Fusarium sp.2 in Ouled Aguile at the second sampling period. Root contamination was also allocated to *Alternaria alternata* detected in all plots for the three visits. In the first isolations this species presented higher frequencies at 17.2% and 17.6% in a small and medium plot respectively whereas in the larger plot, it occurred mostly in the end of growing season. Although less frequent than *A. alternata*, the contamination percentage of Aspergillus sp was high, 22.8 and 26.4% respectively in the third prospection at Ouled Aguile and Gnafda. The other saprophytic fungi *Circinella*, *Penicillium* sp. and *C. herbarum* were detected, these species appeared with frequencies of 0.4% at Gnafda in the first visit, 7.6% at Dlalha in the third one whereas for *C. herbarum* had frequencies of 8% and 4.4% in Ouled Aguile and Dlalha plots respectively. Beside these residents, Phytophothora sp. was isolated in one plot (Dlalha) from roots of the first sampling with a frequency of 15.8%.

The last found competitors in this community were *Trichoderma asperellum* and *Ulocladium* sp. compared to this later one, *T. asperellum* was more frequent reaching percentages of 29%, 26.6 and 37.4% respectively in the third prospecting period of Gnafda, Ouled Aguile and Dlalha while those of *Ulocladium* sp. not exceeding 3.64% recorded in the second visit at Ouled Aguile.

The follow up of the total nematode number and the fungal communities over time retrieved respectively in soil and from roots of strawberry plants cultivated in fumigated plots have displayed noticeable variations.

As for nematode distribution, their population density differs as per the locality and sampling period during which slight changes in soil temperature and soil moisture have been observed. Under soil moisture percentage ranged from 50 to 60%, the number of nematode increased in month of March. According to [43], the increase in nematode population depends upon the season. Mouden [33] found the greatest nematode population density in the fall and the spring. This would be probably due to moisture [44] and ease of movement of the nematodes through the large soil pore diameter and soil particle size [45]. The results presented by Jordaan [43] clearly indicate that multiplication of root knot nematode was found to be highest where soil moisture was also more (18.45%). Likewise, [46] confirmed that higher soil moisture is favourable for nematode multiplication while [47] affirmed that migration and infectivity of *Meloidogyne hapla* is shown to be optimized than parasitism and reproduction of *M. hapla* on strawberry when the soil is moist. Additionally, under soil temperature between 25-30°C, the total number of nematode was much elevated than those recovered at 20-25°C in soil of tomato growing fields in India where soil moisture have been ranged between 4.4% and 18.45% [43]. Indeed, higher temperature causes desiccation

Table 3. Isolation frequencies of fungal species contaminating the roots of strawberry plants cultivated in plots located in Moulay Bousselham during survey time from February to April 2013 (expressed by contamination/ or infection percentage %)

Fungal species	Farm / Visit								
	PS1	PS2	PS3	MS1	MS2	MS3	GS1	GS2	GS3
Alternaria alternata	17.2^{b}	1.7^{c}	17.20^{b}	17.60^{cd}	6.60^{b}	7.20^{bc}	9.40^{c}	1.45^{cd}	17.20^{b}
Aspergillus sp.	0^{c}	0^{d}	26.40^{a}	0^{b}	0^{d}	22.80^{a}	0^{e}	0^{d}	0^{e}
Circinella sp.	0.40^{c}	0^{d}	0^{b}	0^{b}	0^{d}	0^{c}	0^{e}	0^{d}	0^{e}
Cladosporium herbarum	0^{c}	0^{d}	0^{b}	0^{b}	0^{d}	8.00^{b}	0^{e}	0^{d}	4.40^{cd}
Colletotrichum gleoesporioides	0^{c}	3.91^{bc}	0^{b}	12.0^{ab}	3.96^{bc}	0^{c}	0^{e}	0^{d}	0^{e}
Fusarium solani	0^{c}	0^{d}	0^{b}	7.60^{ab}	0^{d}	0^{c}	2.40^{d}	0^{d}	0^{e}
Fusarium sp1	0^{c}	2.21^{c}	0^{b}	0.80 ^{b}	1.24^{cd}	0^{c}	0^{e}	2.64^{bc}	7.60^{cd}
Fusarium sp2	0^{c}	0.49^{d}	0^{b}	0^{b}	3.08^{b}	0c	0^{e}	0^{d}	0^{e}
Penicillium sp	0^{c}	0^{d}	0b	0b	0^{d}	0c	0^{e}	0^{d}	7.60^{cd}
Phytophthora sp.	0^{c}	0^{d}	0^{b}	0^{b}	0^{d}	0c	15.80^{b}	0^{d}	0^{e}
Rhizoctonia solani	34.4^{a}	56.0^{a}	0^{b}	13.60^{a}	18.60^{a}	0c	36.00^{a}	26.72^{a}	0^{e}
Trichoderma asperellum	0^{c}	0^{d}	29.00^{a}	0^{b}	0^{d}	26.60^{a}	0.20^{e}	0^{d}	37.40^{a}
Ulocladium sp.	0^{c}	0^{d}	0^{b}	2.40^{b}	3.64^{b}	0c	0^{e}	3.24^{b}	2,60^{d}

** PS1, PS2, PS3: The three measures separately realized in February (PS1), March (PS2) and April (PS3) at Gnafda parcel.*
MS1, MS2, MS3: The measures realized in February (MS1), March (MS2) and April (MS3) in Dlalha parcel.
GS1, GS2, GS3: The three measures realized in February (GS1), March (GS2) and April (GS3) at Ouled Aguile
Results in the same column followed by the same letter show no significant difference at 5% of probability by LSD test

and dryness of soil and in low soil moisture, nematodes are subjected to increased stress and during this they consume a considerable amount of energy stored and reduce their population density [48].

Nonetheless, the impact of physico-chemical soil properties on nematode population density as pH soil, nitrogen and the organic carbon content is confirmed in previous studies [49,50]. The soil texture, which determines soil compactness and porosity (there by availability of moisture and aeration for the nematodes) is one of the most important soil characteristics related to density of nematode in crop fields [51,52]. On the other hand, the lowest soil infestation level with nematodes observed in the third period in the experimental plot (Gnafda) and that of Dlalha at the second visit would be explained by the short term efficiency of soil fumigant metam sodium (MS) to suppress these organisms. Indeed, many chemical alternatives to MB and their combinations have been evaluated in numerous crops and locations. 1,3-Dicloropropene (1,3-D), chloropicrin (Pic), metham sodium, and their combinations are used for controlling root-knot nematodes and soilborne fungi in greenhouse tomatoes [53,54], cucumbers [55,56], tobacco, pepper, and strawberry [1,57,58,59,60,61] in Italy, the US, Spain, and China.

However, some controversy still remains about the efficacy of these fumigant for nematode management. A significant potential of metam sodium in the suppression of root-knot nematodes (*Meloidogyne* spp.) in French bean under both greenhouse and field conditions was reported [62]. Fumigation with metam sodium was effective for temporarily reducing *Longidorus* population densities before population of nematodes rebounded [63]. This suggests that nematodes can survive in areas where fumigants fail to penetrate, or below the zone of fumigant placement [64]. Nematode control by metam-sodium has been declared to be non-consistent or marginal [65]. Its ineffectiveness is attributed to its mode of action because it is rapidly converted to methyl isothiocyanate which has limited fumigant action and a high affinity for the soil water phase [66]. A significant interaction of soil water content and temperature on the efficacy of metham-sodium against *V. dahliae* microscloretia was previously noted [67].

Results from the studies of [69] show the difficulty in pest-pathogen control for metam sodium (MS) use in Florida sandy soils. Applying metam sodium (MS) either by drip irrigation or by surface spray application followed by soil incorporation led to very erratic field pest-pathogen control. Some MS treated areas exhibited good weed and root knot nematodes control while other areas had intense weed problems and 100% RKN galling of tomato roots [68,69]. In combination with cultural practices or other fumigants, the control of plant parasitic nematodes with metam sodium could be improved [70,71,72]. Greenhouse trials revealed that the blend of 1,3-D and MNa (10+20 g a.i. m−2) greatly inhibited the ability of *Meloidogyne incognita* to form root galls. In addition, the number of colony forming units of *F. oxysporum* declined substantially after growth in media, resulting in higher fruit yields and greater economic benefits [73].

Concerning the distribution and the occurrence of fungal species associated with roots of strawberry plants as expressed by frequency of isolation showed differences as per experimental plot and sampling period. Out of 13 fungal species isolated from roots, the presence of *Rhizoctonia solani*, *Fusarium solani* and the exclusive appearance of *Phytophthora* sp. in one site rejoin the results previously signalled in 7 farms of strawberry production in Gharb and Loukkos region in Morocco [17] where these fungi were registered at higher frequencies than that of *P. cactorum*. These species with *Colletotrichum gloeosporioides* eventually coexist with other soilborne pathogens such *Macrophomina phaseolina*, *Pythium* sp., *Fusarium oxysporum* and causes root and crown rots to strawberry plants [17,74,75,76,77]. In addition, the fungal community examined during the three sampling periods also revealed the existence of *Penicillium* sp., *Aspergillus* sp., *A. Alternata*, *C. herbarum*, *Circinella* sp. and *Ulocladium* sp. no commonly present on roots but more frequent on stems or leaves as study results reported on strawberry plants [11,16] or the olive trees cultivated in the South of Morocco [78]. The four members, *Aspergillus* sp., *Penicillium* sp. *Trichoderma* sp. and *Fusarium* sp. are commonly occurring in soil mycloflora [79]. In Florida, [80] advanced the isolation of *Alternaria*, *Pestalotiopsis* accompanied with *Rhizoctonia*, *Fusarium* spp., *Cylindrocarpon* and *Phoma radicina* from diseased roots of strawberry runner plants from nurseries tested in 2010 and 2012. Rosado-May et al. [81] have detected the presence of *Fusarium, Pythium, Rhizoctonia, Cylindrocarpon, Trichoderma* and *Verticillium* isolated from the strawberry roots.

Nevertheless, the fluctuant occurrence of fungal species throughout the three investigation periods seems to concern all species detected in the studied plots which have received a pre-plant fumigant application prior to planting. Several factors including soil type, temperature, physical properties, pH, and water holding capacity are known to impact the efficacy of metam sodium [82]. Soil temperatures below 10°C will disrupt the generation and dissipation of methyl isothiocyanate [83]. Many workers have found *Trichoderma* and *Penicillium* spp. to be dominant in fumigated soils [84,85]. Saksena [86] studied the resistance of various soil fungi to fumigants and their ability to recolonize the fumigated soils. Similarly, the fungitoxic effect of Formalin and carbon disulphide (CS2 was very pronounced on mycoflora members of sunflower rhizosphere harboring *Aspergillus ruber, A. ochraceus*, *A. luchuensis*, *A. fumigatus, A. niger*, *Penicillium Nigricans, Penicillium funiculosum, Mucor racemosus Trichoderma viride* and *Curvularia lunata* but thereafter they reappeared in the treated soil whereas both *Aspergillus terreus* and *Fusarium oxysporum* were resistant to formalin application [87].

Based on the observed fungi frequency, the relative importance of the four species *F. solani*, *C. gloeosporioides*, *R. solani* greatly decreased at the third visite compared to *T. asperellum*, *A. alternata*, *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp1. This would be related to sensitivity of these fungi towards all control mesures existing or to metam sodium which was applied at the beginning of the 2012-2013 season in the small farm while the medium and big sized farms received fumigation in the preceeding year. Klose et al. [88] revealed that 2735 mmol InLine kg^{-1} soil is needed to kill 90% of *V. dahliae* in this soil. Moreover, among tested species, *Pythium ultimum* was the most sensitive and *V. dahliae* the least sensitive pathogen to fumigation with InLine that also showed an intermediate efficacy for controlling propagules of *F. oxysporum* and *Phytophthora cactorum* in soils [88]. According to [89], incorporation of dazomet in the surface layer and injection of metam-sodium with a polythene cover, resulted in 100% kill of *Fusarium culmorum* and *Pythium* sp., and reductions in *Phytophthora cryptogea, Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

Similarly, previous studies have also reported that fumigation resulted in a change in the soil fungal communities, especially the structure of ascomycetes [90]. Hu P et al. [91] found a differential impact of biofumigant on soil microorganisms. They observed a dramatic decrease in fungal populations (~85% reduction) after allyl ITC addition Also, the fungal community compositions shifted following ITC amendments (e.g., *Humicola* increased in allyl and *Mortierella* in butyl ITC amendments). Bacterial populations were less impacted by ITCs, although there was a transient increase in the proportion of *Firmicutes*, related to bacteria know to be antagonistic to plant pathogens. According to Essarioui and Sedrato [92], the combination of reduced doses of metam sodium with soil solarization resulted in the greatest impact on total fungi and *Fusarium* spp.

Otherwise, Ceja-Torres et al. [93] confirmed that the distribution and prevalence of fungi and pseudo-fungi causing of strawberry dry wilt was influenced by soil texture and the level of organic matter. Also, Bhatti and Kraft [94] demonstrated the effect of the soil moisture on the rhizosphere populations of the wilt and root rot pathogens that increased with an increase in soil moisture content. Indeed the majority of fungal species affecting underground organs of strawberry plants like *R. Solani* [95], *Fusarium oxysporum* [96], *Macrophomina phaseolina* [76], *Colletotrichum* species [97,98] can survive for long periods as resistant structures sclerotia, chlamydospores or as potential inoculum in plant debris and soil [39,99,100]. Under special environmental conditions in rhizosphere, the germination can occur [99,101,102]. It has been hypothesized that survival of *C. acutatum* may improve at lower temperatures [103] as the result of reduced colonization of plant debris by the pathogen whereas it would decrease in the case of increased colonization by other soilborne microorganisms that would compete for nutrients in tissue at high temperatures [103,104]. According to [97], the survival of *C. acutatum* in infected plant debris of leatherleaf fern or in soil increased with the reduction in soil moisture. For *C. gloeosporioides*, the effect of variations in moisture and temperature on its survival in strawberry crowns is unclear [105]. Soil moisture may have affected the survival of *C. gloeosporioides* in buried strawberry crowns by affecting fungus activity or by indirectly disrupting the activity of its competitors [106]. The competitive saprophytic ability of *Trichoderma harzianum* in buried plant tissues is reduced when the soil is flooded or when there is a drastic reduction in the moisture content of the soil [106]. As claimed by [100], in soil, the varying survival capabilities of different types of *Colletotrichum* spp. inoculum is of importance because such inoculum may serve as a potential source for disease outbreak. However under these circumstances the pathogenicity of recovered isolates and the susceptibility of cultivars should be considered.

Researchers approved that soil biodiversity loss and simplification of soil community composition impair multiple ecosystem functions, including plant diversity, decomposition, nutrient retention, and nutrient cycling [107]. Thus, to avoid the destruction of soil ecosystems by many of these chemicals [108,109,110], it is worth noting that management systems that are not dependent on chemical soil fumigation but rely on biologically based approaches are more beneficial Indeed, organic amendments, such as compost, are widely available and offer the advantage of improving soil properties, adding nutrients, recycling wastes [111]. In this sense, composts may enhance plant growth, yield of several crops and suppress plant pathogens by naturally introducing beneficial microbial populations, or by amendment with commercial biocontrol strains [112,113,114,115,116,117,118]. Similarly, [119] indicate that application of antagonists can suppress galling and reproduction of *M.incognita* resulting in enhancement of plant growth. As a ubiquitous soil fungus which colonizes root surfaces and root cortices [120], several species of *Trichoderma*, including *T. harzianum, T. viride*, *T. atroviride*, and *T. asperellum*, have provided excellent control of root-knot nematodes in previous studies [121,122]. The fungal and bacterial isolates (*Trichoderma* and *Bacillus* strains) were able to reduce rootknot nematode damage while increasing yield in crops such as soybeans where no nematicides are currently registered and no rootknot resistant cultivars are currently available in South Africa [123]. In Ethiopia, a effect of different botanicals and *T. harzianum* on individual and in combination for the management of tomato root-knot nematode development and their role on plant growth under greenhouse condition were advantageous [124].

4. CONCLUSION

This is the first survey conducted to estimate the frequency occurrence of fungal flora associated with roots of strawberry plants and the nematode density in interaction with physicochemical

parameters of soil over time at three localities. The current study demontrated the high occurrence of nematodes inhabiting strawberry rhizospheres and the diverse effects of soil physico-chemical properties on their density throughout the three farms and soil sampling periods as well as the variable frequency of fungal communities colonizing roots of strawberry plants cultivated in fumigated soil. Moreover, results showed that even applying metam sodium, among 13 fungal species isolated from roots of strawberry plants, *R. solani* was more prevalent than *F. solani*, *Colletotrichum acutatum* and *Phytophthora* sp. These fungi are known to be capable of causing damage to the crop [125,126,127] with those less harmful. Knowledge of which species is present in a field is important to determine the possible threat to strawberry before to adopt a suitable method that will adequately provide season long nematode and soilborne pathogens control.

The use of special techniques and procedure of application may improve the fumigant action [128]. Therefore, it is necessary that the researchers should pay direct attention towards widespread distribution of nematodes affecting plants, interaction with other soil microorganism and evaluation of their potential damage and influence on crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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