Plant-parasitic nematodes parasitizing saffron in Morocco: Structuring drivers and biological risk identification

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A R T I C L E I N F O

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A B S T R A C T

Plant-parasitic nematodes (PPN) are the most destructive of all plant pathogens. They are an economically important group of soil pathogens, causing significant annual damages of up to 25% of world crop production. Morocco is considered to be a highly productive country for the colorant/medicinal/spice saffron (*Crocus sativus* L.). Taliouine and Taznakht regions are the most productive areas of this valuable neutraceutical. Due to its metabolic profile, and growth forms, saffron is susceptible to many plant diseases, including plant-parasitic nematodes (PPN). This work aims to assess the diversity of PPN communities in soils of Taliouine and Taznakht regions to facilitate understanding of links between their assemblages with biotic and abiotic parameters. Herein, nematode communities were characterized in 163 soil samples collected from 11 rural communes characterized by altitudinal gradients in Taliouine and Taznakht regions. Fifteen PPN genera belonging to 12 families were identified, among which the four genera *Ditylenchus*, *Aphelenchoides, Pratylenchus* and *Helicotylenchus*, potentiate serious limiting factors in saffron production. Their frequencies are respectively 92, 49, 48 and 36% in the area of Taliouine, while in Taznakht they represent 95, 69, 33, and 28% respectively. Regarding the assessment of diversity at different sites, the genus richness (*R*) index ranges from 2 to 10 distinct genera, whereas the Shannon diversity (*H'*) index varies from 0.9 to 1.5 and the Evenness (*E*) index tends to 1. The Co-inertia analyses revealed a substantial relationship between nematode communities and soil types. Soil texture is the major factor influencing the presence and the abundance of a considerable portion of genera. Multivariate analyses (MBPLS) indicated links between humidity, rainfall, minimum temperature and PPN taxa, though maximum temperature did not have an impact. *Ditylenchus, Helicotylenchus, Pratylenchus* and *Paratylenchus* were related to the humidity and silt soil that developed in Taliouine. *Aphelenchoides*, *Tylenchus*, *Tylenchorynchus* and *Dorylaimus* were more prevalent in rainy locations and clay soils of Taznakht. Suitable nematode controlling approaches may be applied and preventative measures should be considered at nursery and field level.

1. Introduction

Saffron (*Crocus sativus* L.) is seen as the most costly and luxurious spice across the globe (Kafi et al., 2006). It has been grown in Morocco for centuries, and has recently gained a great reputation nationally and internationally because of its high quality (Lage and Cantrell, 2009). With the implementation of geographical indication processes in the 'Green Morocco Plan' (Plan Maroc Vert), in 2010, saffron from Taliouine was registered and the region became a protected designation of origin (PDO). This government initiative encouraged an increase in the number of growers between 2010 and 2014 and the production of saffron PDO has improved by 360%. During this period, saffron growers' margin decreased by only 17% for PDO producers whilst it reached 34% for non-PDO producers (Csurgó and Megyesi, 2016). Currently saffron receives considerable attention by farmers who attempt to boost crop production through intensification and monocropping. Unfortunately, intense practices increase the probability of saffron being susceptible to soil bioagressors, including plant parasitic nematodes (PPNs), which are considered the most deleterious parasites (Abtahi and Bakooie, 2017). PPNs induce poor development of stigma and flower production, and decrease the corm yield (Ahrazem et al., 2010). These parasitic organisms inject secretions with a stylet and feed

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on cellular content (Decraemer and Hunt, 2006). Moreover, root decay augments other pathogenic assaults, by fungi, viruses and bacteria, infecting roots (Nasiri et al., 2014). PPN effects on yield losses have been valued as more than 25% of world crop production (Nicol et al., 2011) ranging between 100 to 157 billion USD per year (Ali et al., 2014). Nematological studies were undertaken in the leading saffron producing countries such as Iran (Mahdikhani and Alvani, 2013), India (Sheikh et al., 2017) and Spain (Ahrazem et al., 2010; Ranzenberger et al., 2016). Morocco is a popular producing country of high quality saffron, but there has not been any study addressing the diversity of phytoparasitic nematodes associated with the crop. Consequently, this work aims to generate and develop predictive tools to assess the potential PPN genera that could be considered as biological risk on saffron crops in Taliouine and Taznakht regions. This will contribute to efficient management of pests and increase effective sustainability. This study aims to evaluate the diversity of the PPN communities associated with saffron in the core producing regions in Morocco (Taliouine and Taznakht regions), and to analyse the impact of soil physico-chemical and climatic parameters on their distribution.

2. Materials and methods

2.1. Sampling

The sampling was carried out during March and July 2017 in the Taliouine and Taznakht regions in southern Morocco (Fig. 1). Seven sites were surveyed in the Taliouine region and four in the Taznakht region. The altitude ranges from 1100 to 1965 m in the Taliouine region, and from 712 to 2856 m in the Taznakht region. In Taliouine, the monthly average temperatures are between 10 and 32 °C and annual rainfall is less than 75 mm. In Taznakht, the monthly average temperature is between 1 and 40 ∘C and the annual rainfall is less than 38 mm (ABHSM, 2018).

A total of 163 soil samples were collected from eleven rural communes: Zagmouzen (ZA), Assaisse (AS), Agadir Melloul (AM), Tassousfi (TS), Sidi Hssaine (SH), Askaouen (ASK), Taouyalte (TA) in Taliouine and Ouisselsate (WI), Khousama (KH), Siroua (SI), Iznaguen (IZN) in Taznakht (Fig. 1). The sampled area covers ten percent of the total superficie of saffron production in Taliouine and Taznakht regions. Saffron orchards area is estimated to cover 1630 ha. We sampled the equivalent of 10% which is equal to 163 ha. Each one of this sampled hectare was represented by a composite sample coming from the assemblage of 30 sub-samples, collected in a Zig-Zag pathway with a 2cm-diameter auger at 30 cm depth and placed in a polyethylene bag in order to form a 1kg-reference soil sample. We mixed the 30 sub-samples in order to obtain one homogenized sample. Saffron corms were sampled concurrently. Thirty corms were randomly selected from each saffron orchard.

2.2. Soil analyses

Soil physico-chemical analyses were carried out in the Soil Analysis

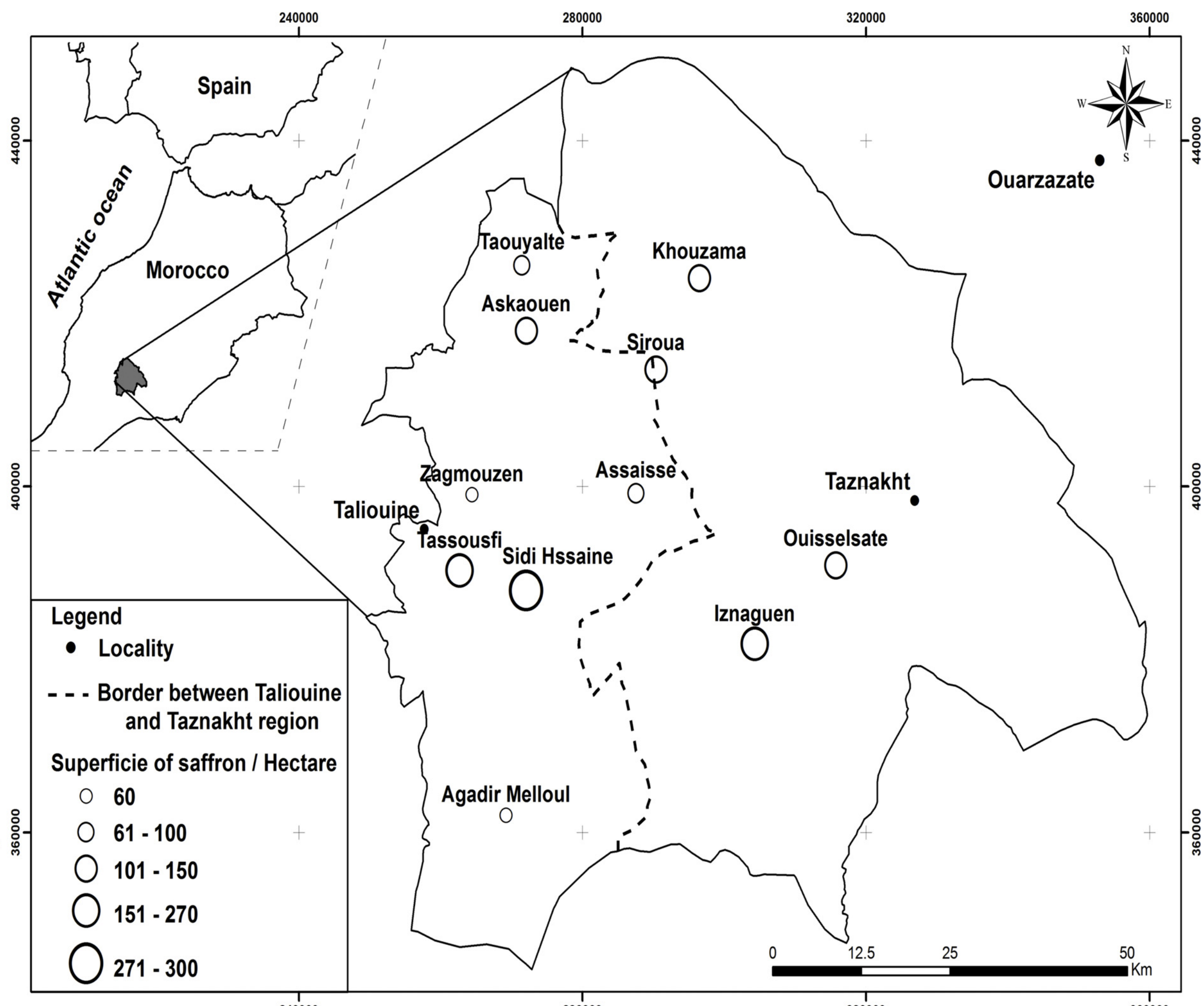

Fig. 1. Map and area of saffron production per hectare in prospected sites of Taliouine and Taznakht regions in the south of Morocco.

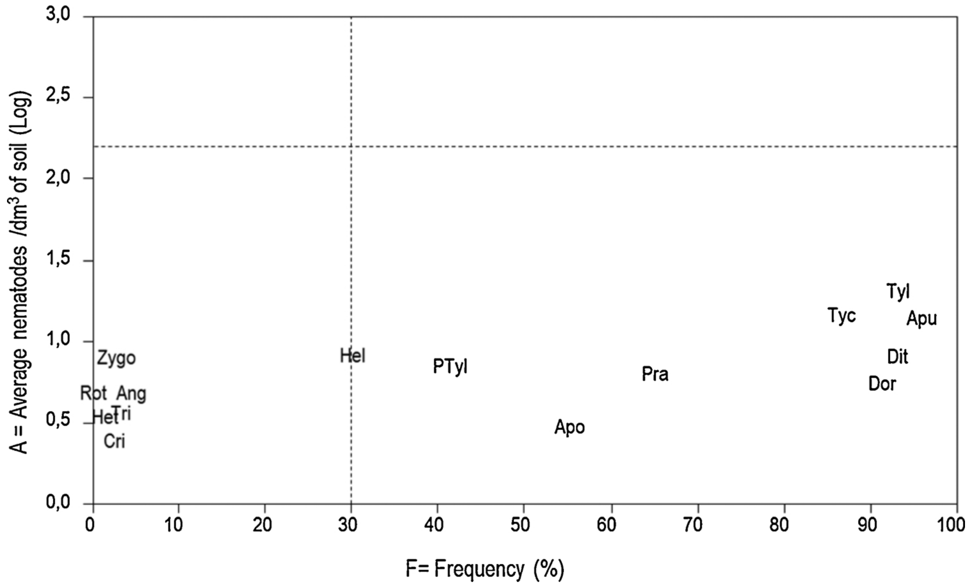

Fig. 2. Dominance diagram of the nematode genera identified in all prospected sites and codes for nematode genera are given in Table 2.

Laboratory of the Agronomic and Veterinary Institute "Hassan II" (IAV, Agadir, Morocco). All soil samples were dried and sieved with a 1 mm mesh. The following soil characteristics were determined: proportion of clays (0–200 µm) and coarse (200–2000 µm) sands according to sedimentation estimation (Hedges and Oades, 1997). The organic matter was estimated by using the method described by Allison, 1960). Total nitrogen was measured using the Kjeldahl nitrogen method (Barbano and Clark, 1990). Contents of assimilated phosphorus, calcium, iron, copper, zinc, sodium, manganese, magnesium, potassium, chloride content, and limestone were measured using atomic spectrophotometry (Lindsay and Norvell, 1978; Sims et al., 1991). pH (H_2O) and conductivity were also measured (Richards, 1954).

2.3. Climatic data collection

Maximum and minimum temperature, humidity and rainfall data for the prospected sites were collected from the Morocco Meteorological department of the hydraulic basin agency of Souss Massa ABHSM (ABHSM, 2018).

2.4. Nematode extraction, characterization and enumeration

Nematodes were extracted from 300 cm^3 of soil by using the normalized elutriation technique (Oostenbrink, 1960; ISO, 2007). PPNs belonging to Aphelenchida (fungal feeders that alternatively feed on plant roots), Dorylaimida, Triplonchida and Tylenchida orders were identified to genus under a stereomicroscope (40X magnification) according to (Mai and Mullin, 1996). PPN population levels were expressed as the number of individuals per 100 cm^3 of fresh soil.

Nematodes were extracted from corms by Baermann methods: a 10 g aliquot of corms and roots from each sample were cut into small pieces and blended in tap water. The suspension was poured onto a paper sieve (50 µM mesh) (Coyne et al., 2010) placed into a plate with water. Forty-height hours after, the nematodes were counted in the filtrate and their numbers were expressed in 10 g of corms.

2.5. Taxonomic diversity

The taxonomic diversity of PPN communities were studied through the following indices: genus richness (G = number of genera in a community); Shannon diversity ($H' = -\Sigma pi*\ln pi$) where pi is the proportion of individuals in each genus; the evenness ($E = H'/\ln G$) that quantifies the regularity of the genus distribution within the community; dominance of genera in samples that was represented by both the frequency (% of samples infested with each genus) and abundance (Log average amount of each genus in all the samples) of each genus (Fortuner and Merny, 1973).

2.6. Statistical analysis

A Principal component analysis (PCA) was used to define the distribution of nematode genera according to their sampling sites. Additionally, a heat map and correlation matrix was produced to check the correspondence between nematodes genera using packages "psy" and "corrplot" (Falissard, 2012; Taiyun Wei and Viliam Simko, 2017) respectively. A dominance diagram of detected genera was applied (Fortuner and Merny, 1973). A Co-inertia analysis (COI) was performed to study the relation between genera and soil physico-chemical factors using ADE4 packages (Chessel et al., 2004; Dray and Dufour, 2007). A k + 1 multivariate method, MultiBlock Partial Least Squares (MBPLS, mbpls (Ade4) (Bougeard et al., 2011) was implemented to underline the interaction between taxa with climatic variables (humidity, rainfall and temperature) and soil variables.

All statistical treatments were executed by the open source software R version 3.2.4.

3. Results

3.1. Diversity of plant-parasitic nematode communities

3.1.1. Diversity assessment of plant parasitic nematodes in soil

In the areas surveyed in the Taliouine and Taznakht regions, PPNs detected in saffron crops belong to 12 families and 15 genera (Fig. 2). *Aphelenchus, Ditylenchus, Tylenchus* and *Tylenchorynchus* were observed in 90% of the field samples. *Criconema*, *Trichodorus* and *Rotylenchus* were exclusively recorded in Taliouine with low frequencies. Among the 15 genera, four genera could be considered as a serious threat for saffron: *Ditylenchus, Aphelenchoides, Pratylenchus* and *Helicotylenchus*. Frequencies were respectively 92, 49, 48 and 36% in the area of Taliouine, whilst in Taznakht they were 95, 69, 33, and 28% respectively. According to the dominance diagrams (Fig. 2) which combine abundance (A) and frequency (F), 40% of the genera were observed to be extensively spread over the prospected location (F ≥ 30%), 40% considered to be rarely seen (F < 5%) and 20% were disseminated and

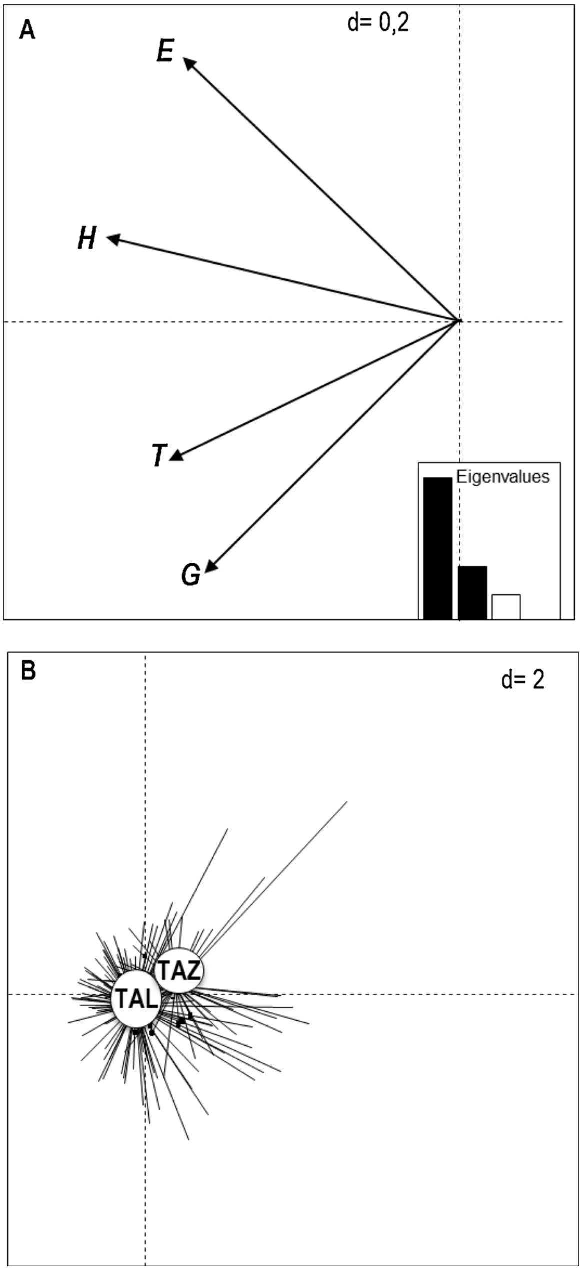

Fig. 3. Principal component analyses of diversity indices: (A) PCA loading plot of the diversity indices; (B) Score plot for the prospected sites of Talioune and Taznakht regions and codes for diversity indices are given in Table 2.

abundant (*Tylenchorynchus* and *Tylenchus*).

The diversity assessment of PPNs associated with saffron in the different sites showed a genus richness (*G*) ranging from 2 to 10 with various genera; whereas the Shannon diversity index (*H'*) varied from 0.9 to 1.47, the highest being seen in Tassousfi and Assaisse and the lowest in Askaouen. The evenness (*E*) ranged between 0.5 to 0.79 for the various sites that indicating genera are widespread across all locations. Plotting PCA showed that all diversity indices were greater in the Talioune region than in the Taznakht region (Fig. 3).

Plotting PCA of the nematode genera exhibited different structures of PPN communities in each region. The first two axes of the PCA accounted for 44.16% of the total variance of the data set (Fig. 4A & B). Taouyalte, Tassousfi and Agadir Melloul communes were partially isolated from the remaining sites. Taouayalte and Tassousfi were partially differentiated by *Helicotylenchus* and Agadir Melloul by *Paratyenchus, Dorylaimus* and *Tylenchorynchus* (Fig. 4C). There was no difference between high, moderate and low altitudes (Fig. 4D).

3.1.2. Diversity in corms

Three PPN genera were recovered from collected saffron corms: *Ditylenchus*, *Aphelenchoides* and *Helicotylenchus* with *Aphelenchoides* being most frequent with high and moderate occurrence respectively in Taliouine (especially in Tassousfi and Askaouen) and Taznakht. Contrastingly, *Ditylenchus* is more frequent in Taznakht and has a lower occurrence in Tailouine. All corms collected from Agadir Melloul (AM) (Ouisselssate) Wi and Taouayalte (TA) were completely free from migratory endoparasitic nematodes (Table 1).

3.2. Co-occurrence between taxa

Heat maps were used to represent the interrelationships between PPN genera. *Dorylaimus* and *Tylenchorynchus* were strongly positively correlated (r = 1). *Tylenchus* were positively correlated with *Aphelenchus, Ditylenchus, Helicotylenchus, Pratylenchus, Tylenchorynchus and Dorylaimus*. Positive correlations were also found between *Aphelenchoides* and *Rotylenchus* (Fig. 5).

3.3. Correspondence between plant parasitic nematodes taxa and pedoclimatic factors

The COI analysis highlighted that the majority of the detected genera were seen to be positively correlated with limestone ($CaCo_3$), potassium (K), manganese (Mn), phosphorus (P_2O_5), sodium (Na), pH and salinity (Ec). The genera were negatively correlated with organic matter (OM), nitrogen (N), copper (Cu), zinc (Zn), iron (Fe), calcium (Ca) and magnesium (Mg) (Fig. 6).

Aphelenchus, Helicotylenchus, Paratylenchus and *Pratylenchus* were strongly associated with silt soils, while *Ditylenchus* was mainly detected in clay soils. The majority of the genera were absent in sandy soils. Where the altitude had no effect on PPNs associated with distribution.

The MBPLS loading plot of the PPN illustrates that *Ditylenchus*, *Helicotylenchus*, *Pratylenchus* and *Paratylenchus* genera were associated positively with the first axis, whereas *Aphelenchoides*, *Tylenchus*, *Tylenchorynchus* and *Dorylaimus* were positively linked to the second axis (Fig. 7A). Climatic factors (Fig. 7B) are rainfall (RF), temperature (T) and humidity (H). The loading plot of the regions (Fig. 7C) showed that the regions are approximately distributed along the first axis. *Ditylenchus, Helicotylenchus, Pratylenchus* and *Paratylenchus* were related to wet and silt soil that occurs in Taliouine. *Aphelenchoides, Tylenchus, Tylenchorynchus* and *Dorylaimus* were present in rainy locations (Taznakht) and clay soils.

4. Discussion

4.1. Taxonomical diversity of plant-parasitic nematodes

During our survey conducted in the south of Morocco in Taliouine and Taznakht regions, we discovered that saffron fields are influenced by environmental, agronomical parameters and nematological risks. These parameters are the major drivers of PPN communities diversity. The composition, frequency, abundance and diversity assessment of PPNs associated with saffron showed variability between and within the different sites surveyed. High diversity indices including genus richness, Shannon diversity and evenness were observed in the Taliouine area especially in Tasoussfi, Zagmouzen, Assaisse and Agadir Melloul sites. Comparatively less diversity was seen in the Taznakht area. This may be explained by the origin of the saffron crop (Tassousfi), the microclimate of each area and soil physico-chemical features (Kandji et al., 2001; Cadet et al., 2005; Karuri et al., 2017).

Plant growth productivity is greatly affected by PPNs (Mateille et al., 2016). Nematodes belonging to the order Tylenchida are

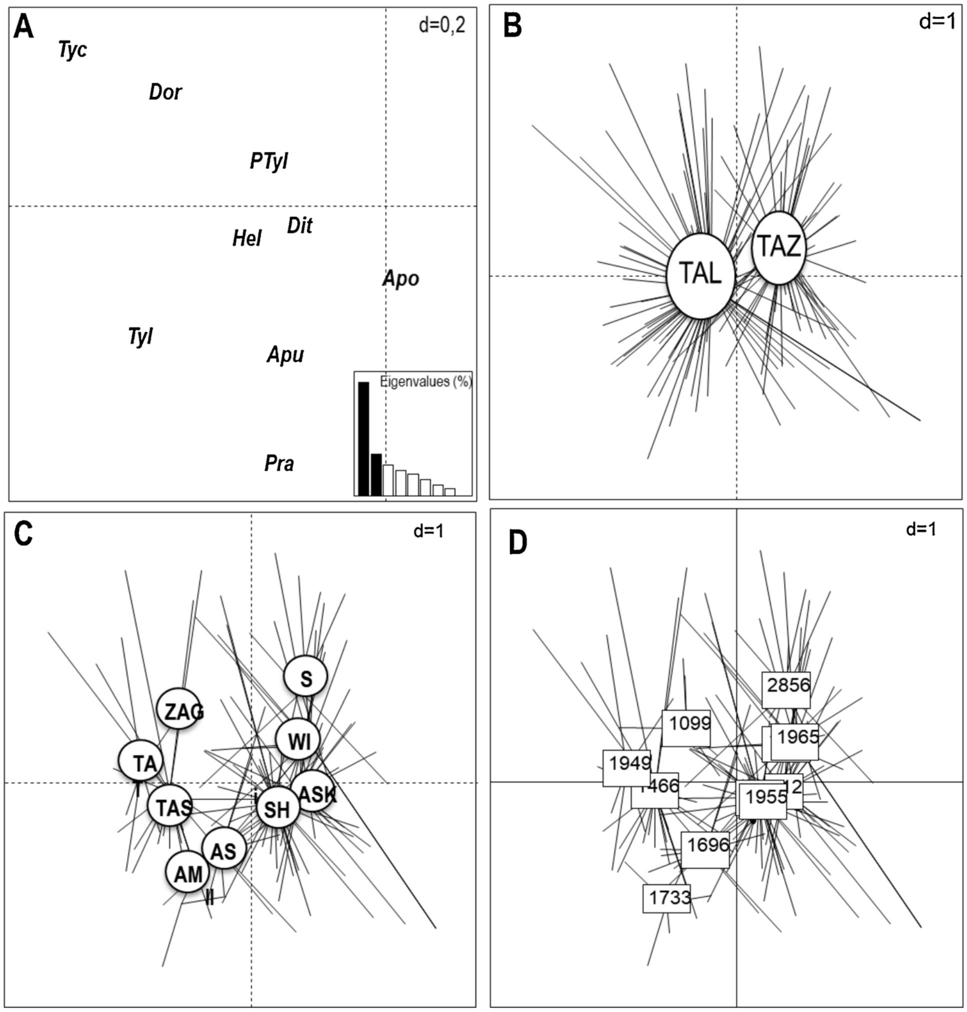

Fig. 4. Principal component analyses of phytoparasitic nematodes genera associated to *Crocus sativus* L.: (A) PCA loading plot of the nematode genera; (B) Score plot for prospected regions; (C) Score plot for prospected sites; (D) Score plot for the altitudinal variation and encoding for the taxa and environmental variables is listed in Table 2.

Table 1
Prospected genera of plant parasitic nematodes in saffron corms collected in differents location from Taliouine and Taznakht regions: Agadir Melloul (AM), Assaisse (AS), Askaouane (ASK), Iznaguene (IZN), Khosama (KH), Ouislsate (Wi), Sidi Hssaine (SH), Siroua (SI), Tassousfi (TS), Taouayalte (TA) and Zagmouzen (ZA); (+ + + : more frequent; + + : frequent; + : less frequent ; - : Absent).

Area	Site	*Ditylenchus*	*Aphelenchoides*	*Helicotylenchus*
Taliouine	SH	+	+	–
	TS	+	+ + +	+ +
	Za	–	+	–
	AS	+ +	–	–
	AM	–	–	–
	ASK	–	+ + +	–
	TA	–	–	–
Taznakht	IZN	+ + +	+ +	–
	Wi	–	–	–
	Kh	–	+ +	+
	Si	+ + +	+ +	–

considered as major agricultural pests (Bernard et al., 2017). Recently the list of economically most important PPNs has been modified by Jones et al. (2013), who reported that the successive species: *Meloidogyne spp.*, *Heterodera* spp. *and Globodera* spp., *Pratylenchus* spp.,*Radopholus similis, Ditylenchus dispaci, Bursaphelenchus xylophilus, Rotylenchulus reniformis, Xiphinema index, Naccobus aberrans* and *Aphelenchoides bessseyi* are the top 10 most damaging PPNs due to their scientific and economic significance. In this study, we detected three genera of PPN in saffron crops of Taliouine-Taznakht. These were *Pratylenchus*, *Ditylenchus* and *Aphelenchoides*, supporting the above findings and constituting the nematological risk to this crop.

The composition of PPNs associated with saffron in the study region of Taliouine-Taznakht is not similar to the genera seen in Khorozan (Iran) (Mahdikhani and Alvani, 2013): *Amplimerlinius, Filenchus, Geocenamus, Merlinius, Psilenchus*. Saffron in the valley of Kashmir (India) (Sheikh et al., 2017) was affected by five genera being *Tylenchus, Helicotylenchus, Pratylenchus, Hirschmaniella, Psilenchus.* In Spain (Ranzenberger et al., 2016) 11 genera were listed, and five of them *(Aphelenchus, Aphelenchoides, Rotylenchus, Helicotylenchus* and *Tylenchus*) were identified within PPN communities in Taliouine-Taznakht.

Among the prospected nematode genera in the soil of Taliouine-Taznakht *Aphelenchoides*, *Ditylenchus*, *Pratylenchus* and *Helicotylenchus* were reported to parasite the saffron crop.

Aphelenchoides spp. and *Ditylenchus* spp. generally attack stems and bulbs as well of saffron crops (Ortuño and Oros, 2002; Vovlas et al., 2011). *Aphelenchoides* has been identified in leaves and corms of *C. sativus* in diverse countries (Decker, 1989; Ortuño and Oros, 2002; McCuiston et al., 2007). *Pratylenchus* has been detected in saffron (Metcalf, 1903; Schenk, 1970), where it causes stunting of plants due to

Table 2
Variables analyzed and corresponding codes.

Variables	Code	Variables	Code	Variables	Code
Nematode genera		*Geographic regions*		*Soil characteristics*	
Aphelenchus	Apu	Taznakht	Taz	sand	San
Aphelenchoides	Apo	Taliuoine	Tal	silt	Sil
Anguina	Ang	Agadir Melloul	AM	Clay	Cla
Criconema	Cri	Askaoune	ASK	Nitrogen	N
Dorlaimus	Dor	Inzagune	IZN	Copper	Cu
Ditylenchus	Dit	Khosama	KH	Iron	Fe
Helicotylenchus	Hel	Ouislsate	WI	Magnesium	Mg
Heterodera	Het	Sidi Hsaine	SH	Manganese	Mn
Paratylenchus	pTyl	Siroua	SI	Phosphorus	P
Pratylenchus	Pra	Tassousfi	TS	Potassium	K
Rotylenchus	Rot	Taouayalte	TA	Sodium	N
Trichodorus	Trd	Zagmouzen	ZA	Zinc	Zn
Tylenchus	Tyu	Altitude	B,C	pH	pH
Tylenchorhynchus	Tyc	*Climate*		Conductivity	Con
Zygotylenchus	Zyg	maximum temperature	T.Max	Organic matter	OM
		of the hottest month (°C)		*Data collection*	
		annual rainfall (mm)	RF	Regional Agricultural Development Office	ORMVA
		Diversity indices			
		total number of nematodes	N		
		species richness	G		
		local diversity	H'		
		evenness	E		

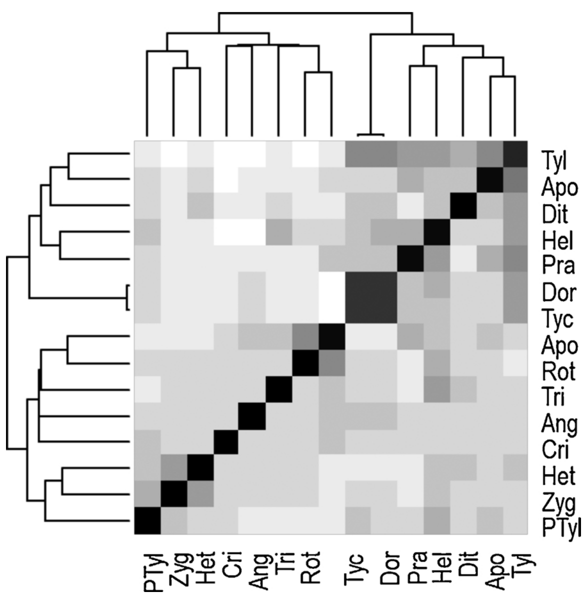

Fig. 5. A heat map between different PPN genera associated with *Crocus sativus* L. and encoding for the taxa is listed in Table 2.

affecting root growth (Jones et al., 2013).

Contrastingly, in this study, our sampled saffron corms were infested by *Ditylenchus Aphelenchoides* and *Helicotylenchus* in the majority of the sites, whilst corms obtained from Agadir Melloul, Ouisselsate and Taouayalte were nematode-free. Such observations may relate to the difference in environmental conditions or land management restricting development of these nematode genera. In addition, no corm infection by *Pratylenchus* was recorded in our study, even though it is widely distributed in soil where saffron crop was cultivated. This suggests the *Pratylenchus* occurring in Taliouine-Taznakht may not be parasitic species of saffron and occurrence is due to habitual cereal crop rotations.

4.2. Co-occurrence between taxa

Positive correlations were evident between nematode taxa in the current study, and may be explained by their synergistic interaction through similar feeding behaviours and persistence (Eisenback, 1993). The existence of a specific species in a site is associated with its propagation habit, an appropriate host, interrelations with other organisms, farming history and other parameters (Norton, 1978; Khan and Khan, 1990; Norton and Niblack, 1991). PPNs often parasitize plants in mixed-species communities (Jones and Perry, 1978). They appear in wide specific communities that are related to their perseverance, polyphagous feeding behaviours, huge distribution and interspecific competition (Oostenbrink, 1966). *Tylenchus* is positively correlated with *Aphelenchus*, *Ditylenchus*, *Helicotylenchus*, *Pratylenchus*, *Tylenchorynchus* and *Dorylaimus*. Interspecific co-occurrence has a driving force via the impact of interaction resulting an increase or inhibition of nematode reproduction (Eisenback, 1993). A further finding of this study is the negative correlation observed between *Tylenchus* and *Zygotylenchus*. These results support the intergeneric inhibition that was reported in previous studies by Ross (1964) and Amosu (1970), whilst the synergystic relationship was discussed by Bird and Jenkins (1964).

4.3. Correspondence of plant-parasitic nematode communities to edaphic conditions

Relationships between the distribution of PPN genera and soil physico-chemical factors were highlighted. The most dominant genera in Talioune and Taznakht regionss were positively correlated with loamy and clay soils, in agreement with previous studies (Salahi et al., 2014; Aït-Hamza et al., 2015; Aït Hamza et al., 2018). High abundances of PPNs were observed in loamy soils whilst clay soils exhibited low PPN abundances. The distribution of *Pratylenchus* is determined by soil texture (McSorley and Frederick, 2002). Moreover, high reproduction of *Pratylenchus* was recorded in clay soils (Grandison and Wallace, 1974; Thompson et al., 2010). A similar trend was reported for *Ditylenchus dipsaci* (Wallace, 1962). In Souss regions *Aphelenchoides, Gracilacus, Pratylenchus,* Rotylenchidae and Tylenchidae tolerated a coarse soil texture (Ferji and Geraert, 1997). Texture is a major factor influencing the occurrence of some species (Cadet and Thioulouse, 1998; Melakeberhan et al., 2018). Soil texture and structure refer to the way soil particles are arranged related to each other and to organic matter. The arrangement of soil particles provides essential information on the properties of a soil and significantly influences the presence of nematodes as the movements of these nematodes depend on soil porosity,

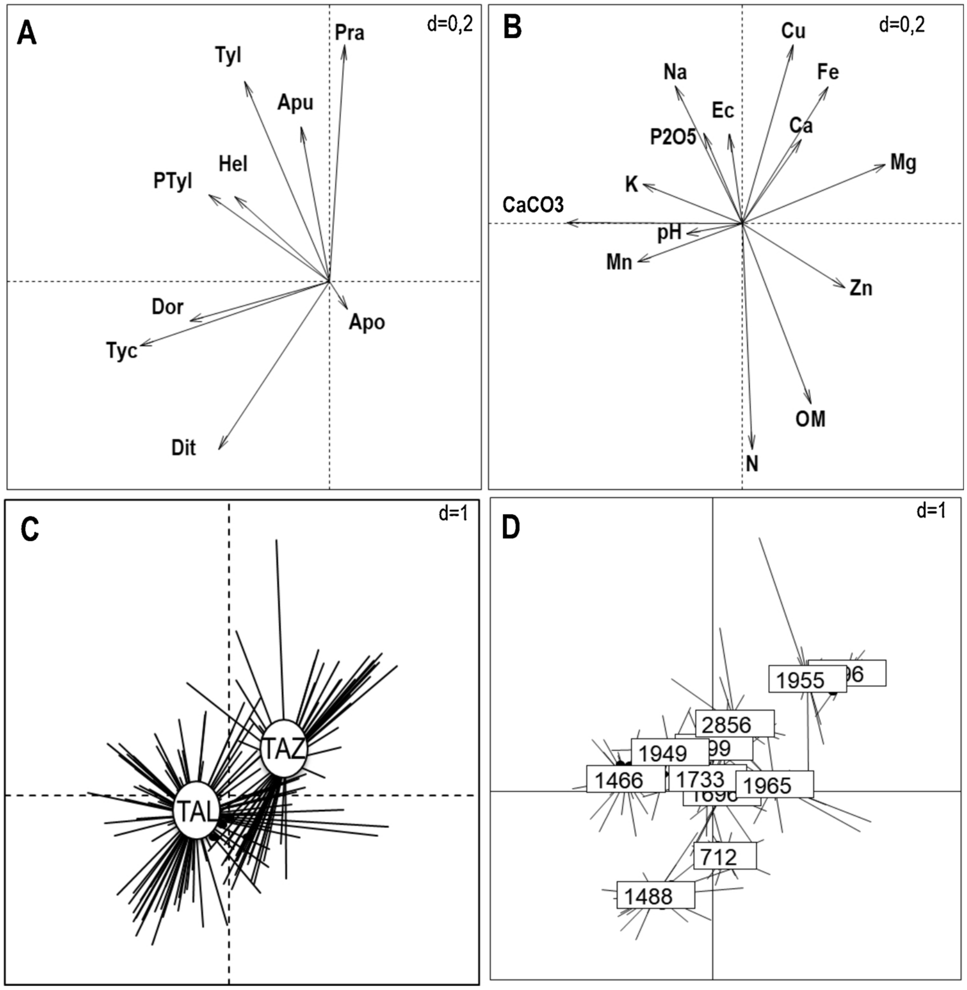

Fig. 6. Co-inertia analyses between PPN and soil physico-chemical characteristics: (A) PCA loading plot of the nematode genera; (B) PCA loading plot of soil physico-chemical characteristics; (C) Score plot for prospected regions; (D) Score plot for the altitudinal variation and encoding for taxa and soil physico-chemical characteristics is listed in Table 2.

size of soil particles, thickness of water films, and the soil's ability to retain moisture (Decker, 1989). In this study, most of the genera showed a negative correlation with organic matter (OM): OM accumulation accounts for a direct decrease of nematode abundance (Hominick, 1999; Qi and Hu, 2007) or PPNs are subjected to microbial antagonisms that increase with OM (Guiran et al., 1980; Widmer et al., 2002). This study confirmed that PPNs abundance is correlated with iron (Fe), calcium (Ca) and sodium (Fiscus and Neher, 2002; Yavuzaslanoglu et al., 2012; Karuri et al., 2017) and is affected by pH and magnesium (Mg) and calcium (Ca) (Cadet and Thioulouse, 1998). Conversely, a positive correlation between *Helicotylenchus* and acidity, magnesium (Mg) and potassium (K) has been reported (Robinson et al., 1987; Zoon et al., 1993).

Most of the genera identified show a negative correlation with total nitrogen (N), except for the *Aphelenchoides*, *Ditylenchus*, and *Criconema*. That can be explained by the accumulation of nitrate and ammoniacal nitrogen through nitrogen degradation that are considered as poisonous to PPNs (Rodriguez-Kabana et al., 1981; Rodriguez-Kabana, 1986). Copper (Cu) and zinc (Zn) cause an adverse impact on nematode community structure, decreasing genus richness, reducing maturity indices of free-living nematodes and reduce the abundance of omnivorous and predatory nematodes (Georgieva et al., 2002). In addition, various soil physicochemical factors have been related to genera and density gradients of some PPNs (Wallace et al., 1993; Chen et al., 2012). Overall, soil textures play a substantial role in the abundance, distribution and structure of PPN communities associated with saffron.

4.4. Correspondences of plant parasitic nematodes to climatic variables

The multiblock analysis showed that *Ditylenchus, Helicotylenchus, Pratylenchus* and *Paratylenchus* were related to the humidity that occurred in Taliouine while *Aphelenchoides, Tylenchus, Tylenchorynchus* and *Dorylaimus* were found in rainy locations of Taznakht. Herein, climatic conditions are the driving force to structure PPN communities particularly through rainfall and humidity (Aït Hamza et al., 2018; Ferreira et al., 2018; Namu et al., 2018). Conversely maximum temperature had an insignificant impact on PPN communities what has been confirmed by Namu et al. (2018). The diversity and the distribution of PPN communities are influenced by temperature and moisture (Neilson and Boag, 1996; Todd et al., 1999; Bakonyi et al., 2007; Nielsen et al., 2014). It should be noted that most of the biological activities of PPN take place at a precise temperature optimum. High temperatures may result in higher nematode reproduction and development (Tzortzakakis and Trudgill, 2005; Evans and Perry, 2009). Moreover, the geographic distribution range of each nematode species is determined according to optimal temperature of its life processes (Luc et al., 2005). The strong relationship between temperature and nematodes distribution (Boag et al., 1991) implies that climate change may affect the distribution of nematodes since climate is linked to the development and spread of various infectious agents. These agents are a source of diseases for agrosystems, plants, free living animals and humans (Harvell et al., 2002; Garrett et al., 2013). The effects on communities could be linked to biological features of nematodes that

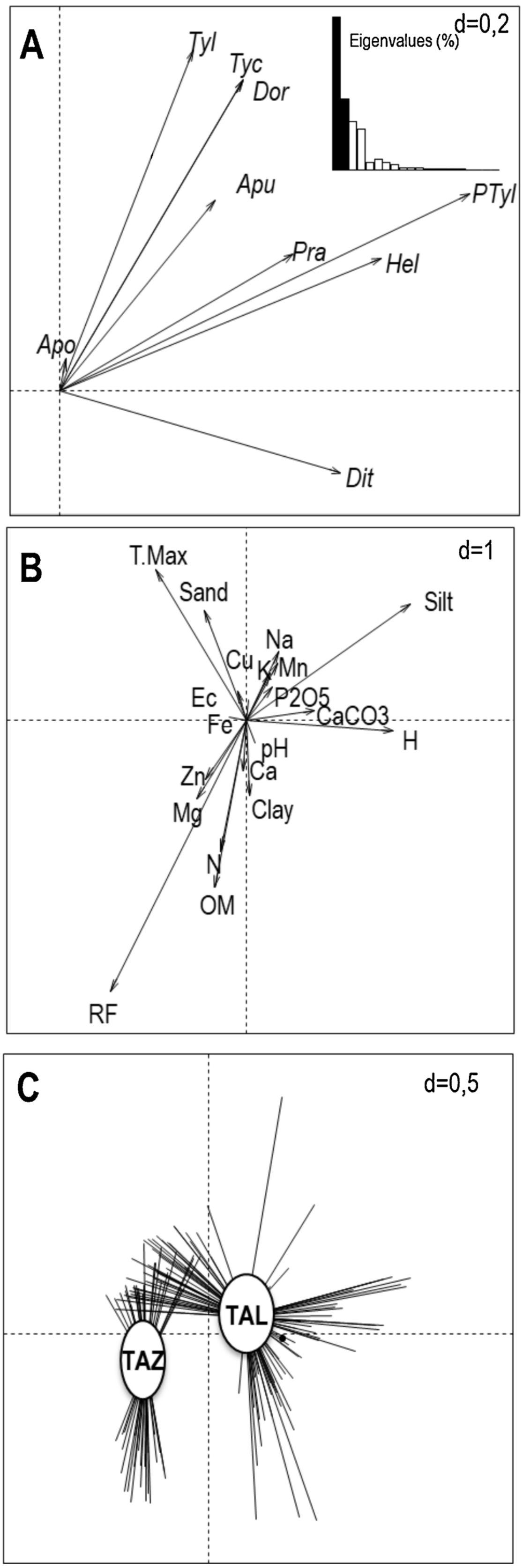

Fig. 7. Multi-block analysis between plant-parasitic nematode communities, soil physico- chemical and climatic parameters: (A) PCA loading plot of the nematode genera; (B) PCA Loading plot of the soil physico chemical and climatic parameters; (C) Score plot for saffron region fields sampled and encoding for taxa and soil physico-chemical characteristics is listed in Table 2.

govern variable reactions to environmental variations and climatic changes. The temperature influences nematode abundance and distribution through effective threats of feeding (Boag, 1980) and root permeation (Roberts et al., 1981). the increased rate of *Pratylenchus* reproduction and development is related to increased temperature (Duyck et al., 2012).The latter is contrary to our results due to *Pratylenchus* species specific characteristics. In this study, most of the genera were related to moisture, which directly affects nematode infection rates. Similarly, the soil dryness influences the survival of PPNs; the lifetime of PPNs in soil looking for root infection is also affected by moisture (Colagiero, 2011). Impacts of temperature on nematode structure, abundance and distribution would additionally drive saffron yield loss.

Saffron crops in Taliouine and Taznakht regions should receive increased attention and intensification according to Morocco's green plan. Consequently, the development of PPNs is improved by land use changes by agriculture, and by agriculture intensification (Mateille et al., 2008). Intensive cultivation methods of conventional crop production degrade soil, and affect biodiversity conservation and agrosystem health sustainability (Lal, 2011). Farmers use traditional techniques and move corms from one field to another. Hence agricultural practices disperse PPN in contaminated corms, soils and materials. As a consequence, the emergence of agronomic risk is increased. This study demonstrated corms infected by *Ditylenchus*, which causes up to 62% yield loss (Latif et al., 2013). Nematodes abundance and diversity are impacted by soil practices and soil texture. Our work supports previous findings that soil physicochemical factors and climatic variables are heavily linked to PPN communities composition and abundance levels (Palomares-Rius et al., 2015; Aït Hamza et al., 2018; Namu et al., 2018).

The outcome of this study provides a basis for developing an agroecological approach to nematode management in saffron production. Consequently, the focus of future investigations is to develop rational measures and suitable agricultural practices that restrict spread and population development of the most damaging PPNs such as *Ditylenchus, Aphelenchoides, Pratylenchus* and *Helicothylenchus*, which should be developed at nursery and field production levels. Further, potential directions for investigations include the clarification of Genotype and Environmental (GxE) expressions of saffron and other species, considering elements of the soil community integrated into GxE expressions to enhance the resolution / accuracy of the GxE result. We make this statement as our results indicate a series of patterns in the behavior of nematodes which may be predicted and further parameterized, we recommend integrating parameterized elements of the soil community in GxE equations (Kearsey and Pooni, 1996), which will achieve a robust / controllable view of plant soil interactions. This study detailed some drivers of PPN communities and further identified discrete species distributions of the soil community though these require further ranking before being placed into hierarchical or synergistic terms for expression within a Markov-chain based tool. Clarification of strategical and functional groups within nematodes and other members of the soil community are required before we proceed with this approach as highly refined input must be fed into Markov chains to obtain accurate results from the tools. Although we have already commenced functional mapping studies of nematodes (Van Den Hoogen et al., 2019), additional recognition of species emergence and ordination within life-history based strategies and continual feeding behavior based functions is required to further the emergence of species in variable environmental settings to ascertain precise order of interactions within predictive tools (Furze et al., 2017; Basaid et al., 2019). The latter will ultimately clarify the risks to production and cultivation of valuable species such as saffron. Such tools give a novel dimension to formulative risk assessment and consider the continued biological exploitation of the soil for human needs. Hereafter, we seek to clarify the nature of physiological causal and effector functions of PPNs and their host species, to show the discrete structure of bioagressors and additional 'passive' members of the soil community. The economic benefits of the use of these 'decision making' tools and their application may revolutionize investigations and use of biological resources into the indefinite future.

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