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Phytosanitary problems in elephant garlic (*Allium ampeloprasum* var. *holmense)* in the "Val di Chiana" area (Central Italy), and evaluation of potential control strategies

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Summary. *Allium ampeloprasum* var. *holmense* (elephant garlic) is traditionally cultivated in "Val di Chiana", an area between Umbria and Tuscany regions of Central Italy, under the name "Aglione della Valdichiana". This product has recently increased in importance, becoming a key economic resource for local farmers. In 2019, phytosanitary problems of elephant garlic cloves ready for transplanting emerged in this cultivation area. Symptom/sign observations and fungal isolations were performed for cloves divided into four components (tunic, basal plate, reserve tissue and shoot) from six farms in the "Val di Chiana" area. Isolates obtained were identified, using partial *β-tubulin* (*BenA*) and *calmodulin* (*CaM*) or *translation elongation factor 1α* (*tef1α*) genes sequences, as belonging to *Penicillium* [*P. allii* (95%), *P. citrinum* (4%), *P. brevicompactum* (1%)] or *Fusarium* [*F. oxysporum* (81%), *F. proliferatum* (19%)]. *Fusarium* spp. were mainly associated with clove tunics and basal plates, while *Penicillium* spp. with basal plates, reserve tissues and shoots. Fungi often also developed from asymptomatic components, but a correlation was found between isolated pathogens and disease symptoms. Pathogenicity and virulence towards elephant garlic cloves were verified for a representative isolate of each identified species, and *Penicillium allii* was the most virulent. Strategies to control *Fusarium* and *Penicillium* spp. on cloves were assessed, including chemicals, a biocontrol agent, surface sterilization and heat treatment. Among these, treatments with Patriot Gold® (active ingredient [a.i.] *Trichoderma asperellum* TV1, approved in organic farming on crops similar to elephant garlic), or Signum® (a.i. boscalid + pyraclostrobin, approved for Integrated Pest Management systems on crops similar to elephant garlic), were effective in simultaneous reduction of *Penicillium* spp. and *Fusarium* spp. Transplanting of asymptomatic cloves combined with the use of the above treatments showed promising effects for pathogens control, and to assist elephant garlic crop establishment.

Keywords. Bulb diseases, *Fusarium*, *Penicillium*, *Trichoderma*, disease management.

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INTRODUCTION

Allium (*Amaryllidaceae* J.St.-Hil., subfamily *Allioideae* Herb) is a broad genus including many bulbous species (Han *et al.*, 2020). Within *Allium*, the species complex *Allium ampeloprasum* L. has wide distribution in the Mediterranean area. The important species *Allium ampeloprasum* var. *porrum* (L.) J. Gay (leek) and *A. ampeloprasum* var. *holmense* (Mill) Asch. et Graebn (commonly referred as elephant garlic) (Guenaoui *et al.*, 2013; Ascrizzi and Flamini, 2020; Terzaroli and Caproni, 2020) are included in the *A. ampeloprasum* species complex.

Allium spp. are appreciated for their health benefits as they contain bioactive compounds with antifungal, antibacterial, antiviral, antitoxic and anticancer properties (Keusgen *et al.*, 2006; Kim *et al.*, 2018; Ceccanti *et al.*, 2021). Among these compounds, the sulfur-containing substances (i.e. alliin and its derivatives) have antimicrobial activities, and are also responsible for human smell, taste, poor digestion and bad breath (Borlinghaus *et al.*, 2014). The very low amounts of alliin and related derivatives present in elephant garlic make these bulbs potential cuisine substitutes for common garlic (*Allium sativum* L.), because their flavour is very close to that of common garlic, but with milder impacts on human breath and digestion (Block, 2011; Ascrizzi and Flamini, 2020; Ceccanti *et al.*, 2021).

Allium ampeloprasum var. *holmense*, native to the Mediterranean basins, is cultivated in many other regions (Fritsch and Friesen, 2002; Guenaoui *et al.*, 2013). It has oversized bulbs and large cloves (Guenaoui *et al.*, 2013). In Italy, elephant garlic is mainly cultivated in "Val di Chiana", a valley between Umbria and Tuscany Regions (Central Italy), as a local landrace named "Aglione della Valdichiana" (Terzaroli *et al.*, 2022). In this territory, elephant garlic was cultivated in family gardens by elderly farmers, and was very close to extinction (Terzaroli, 2015; Terzaroli and Caproni, 2020). However, recent expansion of demand for this product had led to cultivation in "Val di Chiana", and, at the same time, many imitations on the market. For this reason, "Aglione della Valdichiana" has been included in several regional and national traditional product lists (Ministry of Agriculture, Food and Forestry Policies, 2016; Tuscany Region, 2016; Umbria Region, 2020; Slow Food Foundation, 2023). The "Association of Manufacturers and Transformers of Aglione della Valdichiana" is also attempting to obtain the "Protected Designation of Origin" award (PDO) for elephant garlic.

Despite this increasing interest in elephant garlic cultivation, little is known about phytopathological problems of bulbs caused by fungal pathogens, and related disease control strategies. Since elephant garlic is vegetatively propagated due to inability to produce seeds (Terzaroli *et al.*, 2022), bulb phytosanitary status is important during propagation, as well as for commercialisation. As for other *Allium* spp., the health of cloves/bulbs can be compromised by fungal pathogens, including *Fusarium* and *Penicillium* (Dugan *et al.*, 2011; Le *et al.*, 2021). For example, in Chile, *P. hirsutum*, *P. aurantiogriseum*, *P. echinulatum*, *P. funiculosum* and *P. rugulosum*, and *F. oxysporum*, were isolated from stored elephant garlic bulbs showing lesions (Besoain *et al.*, 2002). In addition, *F. proliferatum* has been isolated from rotten elephant garlic cloves in Serbia (Ignjatov *et al.*, 2019). *Fusarium* and *Penicillium* are also well-known causal agents of bulb rot of common garlic (Crowe, 1995; Valdez *et al.*, 2006; Valdez *et al.*, 2009; Gálvez *et al.*, 2017a; Chrétien *et al.*, 2020; Gálvez and Palmero, 2021; Mondani *et al.*, 2021a).

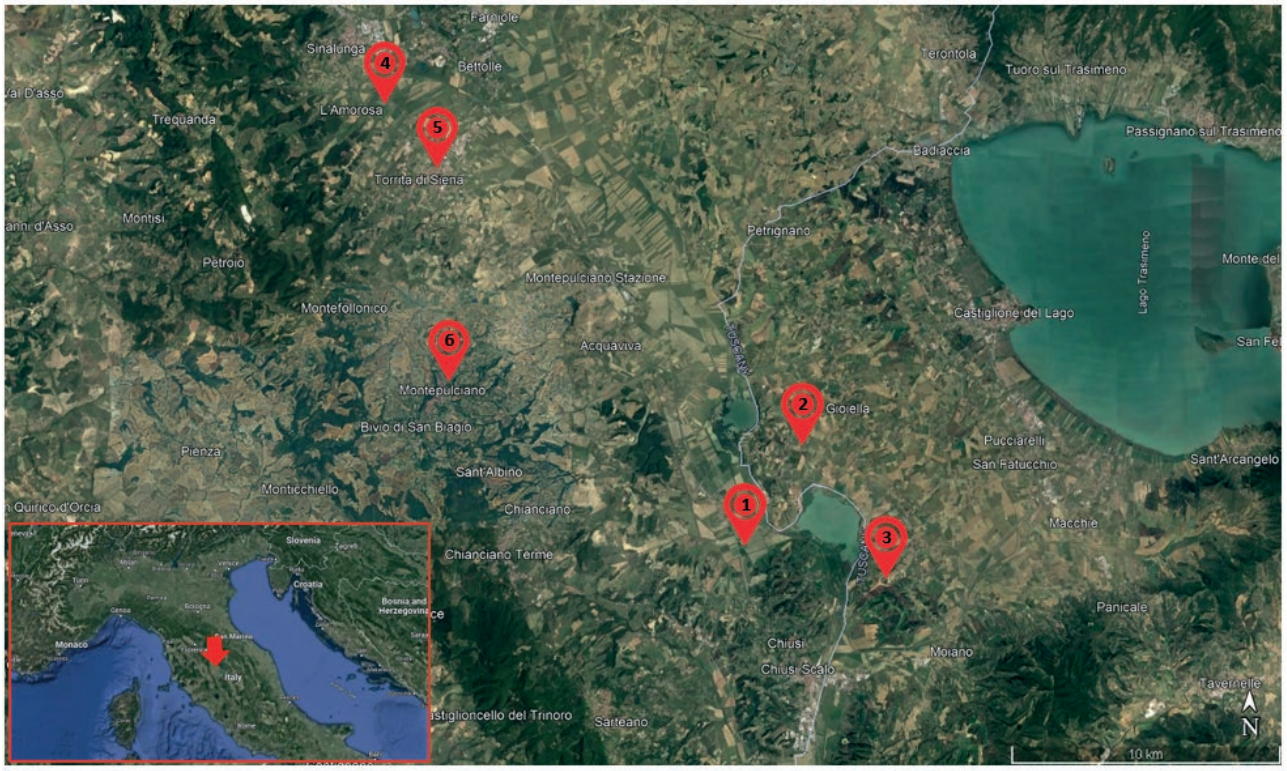
To date, while reports are available on treatments to control *Fusarium* (Dugan *et al.*, 2007; Palmero Llamas *et al.*, 2013; Gálvez *et al.*, 2017b; Mondani *et al.*, 2021b; 2021c; 2022) and *Penicillium* (Greathead, 1978; Bogo, 1997; Johnson, 2013; Ciabanal *et al.*, 2021) in common garlic, no strategies have been tested or developed to control these pathogens on elephant garlic cloves.

Symptoms and signs of fungal infections on elephant garlic cloves ready for planting have been reported in 2019 from several farms of the "Val di Chiana" area in Tuscany and Umbria (Central Italy). For this reason, the present study aimed to: 1) observe the distribution and characteristics of the symptoms/signs in elephant garlic cloves; 2) determine the fungal community associated with components of elephant garlic cloves by isolation into culture; 3) confirm identification of the fungi with molecular methods; 4) assess their pathogenicity and virulence to elephant garlic cloves; 5) test different potential clove treatments for control of clove pathogens.

MATERIALS AND METHODS

Sampling and symptom/sign observations

This study was carried out on elephant garlic cloves collected from six farms located in the "Val di Chiana" area (Figure 1). After harvest (June 2019), the cloves were stored at each farm using the common farming practices. At the time of transplanting (September 2019), 50 cloves were randomly sampled from each farm, from cloves already selected by the farmer for transplanting, to give a total of six samples. These were



Sample	Location	Province	Region	Previous crop	Harvest season	Presence of other *Allium* spp. in the crop system
1	Montallese	Siena	Tuscany	Alfalfa	2019	No
2	Porto	Perugia	Umbria	Tomato	2019	No
3	Villastrada	Perugia	Umbria	Sunflower	2019	Yes (common garlic)
4	Sinalunga	Siena	Tuscany	Wheat	2019	No
5	Torrita di Siena	Siena	Tuscany	Barley	2019	No
6	Montepulciano	Siena	Tuscany	Grassland	2019	No

Figure 1. Map showing the six sampling locations in "Val di Chiana" area, a valley in the Umbria and Tuscany regions of Central Italy. The inset (bottom left) shows a map indicating (red arrow) "Val di Chiana" location. The table presents details relating to each sample location.

brought to the laboratory and 20 cloves per sample were randomly selected. Each clove was divided into four components: tunic (outer husk); basal plate (the attachment point of roots in the bulb); reserve tissue (fleshly part of the clove); and shoot (the shoot primordium within each clove). A sterilized blade was used for each clove dissection to avoid contamination. After defining the symptom/sign categories observed in the cloves, incidence (% of symptoms/signs for each clove component on each sample) was assessed and expressed as the average for 20 cloves. In addition, incidence (% of symptoms/signs per clove component) on all the cloves analysed in the survey was calculated and expressed as the average for 120 cloves.

Determination of the fungal community associated with elephant garlic cloves

The different components (tunic, basal plate, reserve tissue and shoot) of the cloves used for symptom/sign observation were used for isolations of fungi, using the method of Beccari *et al.* (2018). From symptomatic cloves, isolations were made by taking portions showing signs or symptoms, while, for the asymptomatic cloves, isolations were made from portions of the different clove components. Each clove component was disinfected for 1 min by immersion in a solution of 95% ethanol (Sigma Aldrich) + 7% sodium hypochlorite (Carlo Erba Reagents) solution (82:10:8 % vol.), and was then rinsed

three times in sterile deionized water. After disinfection, each component was cut into seven pieces (approx. 2 × 3 mm) that were placed into a Petri dish (90 mm diam., Nuova Aptaca) containing potato dextrose agar (PDA, Biolife Italiana) amended with streptomycin sulphate (0.16 g L^{-1}, Sigma Aldrich). From each clove, four Petri dishes (one per component) were used, giving a total of 80 Petri dishes per original component sample and 480 for the entire survey. The Petri dishes were incubated at 22 ± 2°C in the dark. After 5 d of incubation, a combination of a visual, stereomicroscope (SZX9, Olympus) and microscope (Axiophot, Zeiss) observations were carried out on each Petri dish, to examine development of fungal colonies and to ascribe these to fungus genera.

Incidence (%) of each fungal genus was calculated. Because fungi from cloves were mainly from *Fusarium* or *Penicillium*, the averages of isolates belonging to these genera in each clove component were compared. In addition, for each sample, the number of *Fusarium* and *Penicillium* colonies isolated from each clove component was compared to the numbers isolated from the other components. The average number of *Fusarium* and *Penicillium* isolates obtained from each clove component for each sample was also assessed.

Identification of Fusarium *and* Penicillium *isolates*

Based on the above observations, *Fusarium* and *Penicillium* were the most frequently isolated genera, so isolates potentially belonging to these genera were identified by molecular means, adapting the method described by Beccari *et al.* (2020). All *Fusarium* and *Penicillium* colonies were transferred from isolation PDA cultures into new Petri dishes (one dish per isolate), and these were incubated in the dark at 22 ± 2°C for 10 d. Resulting colonies were assigned to morphotypes according to their shapes and colours, detected visually, and morphology of conidiophores and conidia, detected by optical microscope (Axiophot, Zeiss). A subset of representative isolates was chosen, one from each morphotype. For each representative isolate, a monosporic culture was obtained on PDA and incubated in the dark at 22 ± 2°C. After 10 d, mycelium was scraped from PDA in each dish and placed into a 2 mL capacity sterile plastic tube (Eppendorf) at -80°C, lyophilized with a Heto Powder Dry LL3000 freeze-drier (Thermo Fisher Scientific), and reduced to a fine powder with a Mixer Mill MM400 (Retsch) set at frequency of 25 Hz for 6 min.

DNA extraction was carried out using the methods of Covarelli *et al.* (2015) and Beccari *et al.* (2018). Genomic DNA was quantified using a Qubit® 3.0 fluorometer (Thermo Fisher Scientific), using the dsDNA Broad Range Assay kit (Thermo Fisher Scientific), following the manufacturer's protocol. Each DNA sample was adjusted to a concentration of 30 ng μL^{-1} adding sterile water for molecular biology (5prime). DNA extracts were subjected to partial *translation elongation factor 1α* (*tef1α*) gene amplification and sequencing for *Fusarium* isolates (O'Donnel *et al.*, 1998; Geiser *et al.*, 2004), or partial *β-tubulin* (*BenA*) and *calmodulin* (*CaM*) gene amplification and sequencing for *Penicillium* isolates (Houbraken and Samson, 2011; Visagie *et al.*, 2014). The primers used in the PCR assays are shown in Table S1. Each PCR protocol used a total reaction volume of 50 µL. Each reaction contained 29 µL of sterile water for molecular biology, 5 µL of dNTPs mix 10 mM (Thermo Fisher Scientific), 2.5 µL 10× Dream Taq Buffer + magnesium chloride (Thermo Fisher Scientific), 3.75 µL of cresol red (Sigma Aldrich), 2.5 µL of 10 µM of primers, 0.25 µL of 5 U μL^{-1} Dream Taq Polymerase (Thermo Fisher Scientific), and 2 µL of template DNA (≈60 ng DNA). The PCR cycle consisted of an initial denaturation step (94°C for 5 min), followed by 30 cycles of denaturation (94°C for 1 min), annealing (1 min at the temperature shown in Table S1), extension (72°C for 1 min), and final extension (72°C for 10 min). PCR assays were carried out on a T-100 thermal cycler (Bio-Rad). PCR fragments were visualized on TAE 1X agarose gel (2%) containing 500 µL L^{-1} of RedSafe™ (4% v/v) (Chembio). DNA fragments were separated at 110 V for ≈40 min and observed with a gel documentation system (Essential V6, Uvitec). The sizes of the amplified fragments were obtained by comparison with HyperLadder 100-1000 bp (Bioline Meridian Bioscience).

PCR fragments were purified and sequenced by a commercial service (Genewiz Genomic Europe, Leipzig, Germany). The sequences obtained were verified and edited by Chromatogram Explorer Lite v4.0.0 (Heracle Biosoft srl 2011), and were compared with those deposited in the NCBI Basic Local Alignment Search Tool (BLAST) database (Altschul *et al.*, 1990).

Phylogenetic analyses were carried out for *Fusarium* and *Penicillium* isolates using *tef1α* (O'Donnel *et al.*, 1998; Geiser *et al.*, 2004; Southwood *et al.*, 2012; Taylor *et al.*, 2016; Crous *et al.*, 2021) for *Fusarium*, or *BenA* and *CaM* partial genes sequences (Houbraken and Samson, 2011; Visagie *et al.*, 2014) for *Penicillium*. The sequences of the *Fusarium* or *Penicillium* representative isolates obtained in the present study were analyzed together with those of validated phylogenetic species reported in GenBank (Tables S2 and S3), using MEGA software version 7.0 (Kumar *et al.*, 2016). *Fusarium redolens* NL_96 (Taylor *et al.*, 2016) was used as the outgroup for *Fusarium* phylogeny, and *Talaromyces flavus*

isolate CBS 310.38 (Houbraken and Samson, 2011) was used as the outgroup for *Penicillium* phylogeny. After sequence alignments, nucleotide gaps and missing data were deleted and phylogenetic trees were built using the neighbor-joining method (Saitou and Nei, 1987) with the bootstrap test for 1000 replicates (Felsenstein, 1985). The maximum composite likelihood method (Tamura *et al*., 2004) was used to compute the evolutionary distances.

Pathogenicity and virulence tests

Pathogenicity and virulence tests were carried out using one representative isolate each of *F. proliferatum* (isolate F 88), *F. oxysporum* subclade 1 (isolate F 129), *F. oxysporum* subclade 2 (isolate F 125), *F. oxysporum* subclade 3 (isolate F 42), *Penicillium allii* (isolate P 104), *Penicillium citrinum* (isolate P 41), and *Penicillium brevicompactum* (isolate P 150). Pathogenicity and virulence were evaluated using the methods of Dugan *et al.* (2007) and Ignjatov *et al*. (2019), with slight modifications. Asymptomatic elephant garlic cloves obtained shortly after harvest were disinfected by dipping for 30 sec in water-ethanol-sodium hypochlorite solution (see above), and rinsed three times with sterile water. Cloves were each wounded in two sites by a sterile cork-borer producing wounds of 4 mm depth and 5 mm width. Each wound was filled with a mycelium plug (5 mm diam.) taken from a 7-d-old colony grown on PDA in the dark at 22 ± 2°C. For each fungal isolate, four cloves (replicates) were inoculated, for a total of 32 cloves. In addition, four cloves were used as inoculation controls and were treated with sterile PDA. Inoculated and control cloves were placed in separate transparent plastic trays, which were sealed to maintain a 100% moisture, and were then incubated in a climatic chamber (F.lli Bertagnin) at 22°C, 16 h daily photoperiod. On each clove wound, symptoms were assessed at 30 d post-inoculation (dpi), as the average diameter of dry rot lesion (cm), calculated as the mean of two diameters perpendicular to each other. In addition, to obtain the virulence indices (VIs), average lesion diameter was multiplied by the average wound depth (cm), given by the mean of the depth of the two lesions for each clove, each measured with a ruler. For each isolate, the VI index expressed its aggressiveness (virulence), as the average of the four replicates (cloves).

Evaluation of control of Fusarium *and* Penicillium *spp. on elephant garlic cloves*

A pot trial evaluated different treatments applied to cloves at planting for efficacy against *Fusarium* and *Penicillium* infections. Treatments applied are listed in Table 1. These included: sodium hypochlorite disinfection (2% NaOCl), either alone or with heat treatment (50°C/30 min) in a water bath, also followed by the application of Patriot Gold®, a commercial preparation of *Trichoderma asperellum* strain TV1. Other treatments included: Patri-

Table 1. Treatments applied to elephant garlic cloves for the control of *Fusarium* spp. and *Penicillium* spp.

Treatment	Application	Company	Active ingredient	Dose	Notes
Untreated control	-	-	-	-	-
Sodium hypochlorite (2%)	Immersion	-	Sodium hypochlorite	-	Immersion for 3 min in a water-sodium hypochlorite (2%) solution
Sodium hypochlorite (2%) and heating	Immersion + heating	-	Sodium hypochlorite	-	Immersion for 3 min in a water-sodium hypochlorite (2%) solution, followed by immersion in water for 30 min at 50°C
Sodium hypochlorite (2%), heating and Patriot Gold®	Immersion + heating + dressing	Sumitomo Chemical	Sodium hypochlorite + *Trichoderma asperellum* (TV1 strain, 1×10^6 UFC g^{-1})	0.01 g 6 mL^{-1}	Immersion for 3 min in a water-sodium hypochlorite (2%) solution followed by immersion in water for 30 min at 50°C, and treated with Patriot Gold® (see below).
Patriot Gold®	Dressing	Sumitomo Chemical	*Trichoderma asperellum* (TV1 strain, 1×10^6 UFC g^{-1})	0.01 g 6 mL^{-1}	For each treatment, cloves were placed in a plastic bag containing the product. The plastic bags were gently shaken for 3 min to promote the maximum contact between the product and the cloves.
Signum®	Dressing	BASF	Pyraclostrobin (6.7 g) + boscalid (26 g)	0.09 g 6 mL^{-1}	
Celest Trio®	Dressing	Syngenta	Fludioxonil (25 g L^{-1}) + difenoconazole (25 g L^{-1}) + tebuconazole (10 g L^{-1})	4 mL 40 mL^{-1}	

ot Gold®, without previous hypochlorite and heat treatments, and the fungicides Signum® (pyraclostrobin + boscalid), or Celest Trio® (fludioxonil + difenoconazole + tebuconazole), applied at label rates by shaking cloves in solutions for 3 min, followed by 24 h at room temperature to dry (Table 1). Patriot Gold® and Signum® are currently registered in Italy for use on common garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and leek (*Allium ampeloprasum* L.). Celest Trio® is registered in Italy for treatment of cereal seeds against *Fusarium*. Each treatment, including an untreated control, consisted of ten asymptomatic cloves (ten replicates). Each clove was planted in sterile peat in a plastic pot (8 × 8 × 9 cm) and maintained in a growth cabinet for 30 d at 22°C with a 15 h light 9 h dark daily cycle. At 30 d after the treatments, clove germination rate (%), shoot length (cm), shoot fresh weight (g) and shoot dry weight (g: obtained by placing the shoots in a drying oven at 50°C for 48 h) were measured. In addition, to evaluate effects of the different treatments on frequencies of *Fusarium* spp. and *Penicillium* spp. infections, fungal isolations were carried out for each clove from the four different components (tunic, basal plate, reserve tissue, and shoot) following the method described above, but because the cloves were sprouted, the first centimetre of each seedling was taken for the isolation procedure. Frequencies of isolations (n) of *Fusarium* spp. and *Penicillium* spp. were recorded.

Statistical analyses

All data were subject to one-way analysis of variance (ANOVA). In all cases, Tukey Honestly Significant Difference (HSD) ($P \leq 0.05$) was used to assess pairwise treatment contrasts. All statistical analyses was carried out using Microsoft Excel Macro "DSAASTAT" ver. 1.0192 (Onofri and Pannacci, 2014).

RESULTS

Symptoms and signs on elephant garlic cloves

Different symptoms and signs were observed on the clove components (tunic, basal plate, reserve tissue, or shoot) of the six samples analysed (Table 2, Figure 2). On tunics (Table 2, Figure 2, a, b, and c), pink-purple spotting was detected on average on 28% of the 120 cloves analysed, and browning was detected on 45% of these cloves. Asymptomatic tunics were also detected (27%). Considering the average of the whole survey, no statistically significant differences ($P > 0.05$) were detected between pink-purple spotting, browning and asymptomatic tunics. On basal plates (Table 2, Figure 2, d, e, and f), grey mould signs (35% of cloves) were the most commonly detected, followed by symptoms of sponge-like rot (23%) and pink mould signs (13%). Of total cloves analysed 29% had asymptomatic basal plates. Also on the basal plates, no differences ($P > 0.05$) were detected between the different symptom/sign categories recorded. On reserve tissues (Table 2, Figure 2, g, h and i), rot symptoms (soft watery, or dry) were very common (87% of the total number of cloves analysed and 100% of samples 2, 3 and 6). Black-purple streaks (7%) or asymptomatic tissues were less frequent (present only in sample 5). In this clove component, incidence (%) of rot symptoms was greater ($P \leq 0.05$) compared to black-purple streaks or no symptoms. On shoots (Table 2, Figure 2, j, k, and l), basal grey mould (15%) was the most detected sign followed by dry rot symptoms (3%) and white mould signs (2%). Asymptomatic shoots were very common, detected in 81% of the total cloves analysed, more ($P \leq 0.05$) than for samples with basal grey mould, dry rot or white mould.

Fungal communities associated with elephant garlic cloves

After 7 d of incubation on PDA, from all the clove components of the six clove samples (480), 66% of the total isolated fungi were morphologically identified as *Penicillium*, and 32% were identified as *Fusarium* (Figure 3 a). Fungi not included in these two genera ("other genera"), were isolated with incidence of 2% (Figure 3 a).

The average number of fungal isolates (n) belonging to *Fusarium* and *Penicillium* per analysed clove component are shown in Figure 3 b. The average number of *Fusarium* isolates was greater ($P \leq 0.05$) than *Penicillium* only on the clove tunics. For the other clove components (basal plates, reserve tissues, shoots) greater numbers ($P \leq 0.05$) of *Penicillium* isolates than *Fusarium* isolates were obtained (Figure 3 b). The distribution of *Fusarium* across the four clove components showed the following gradient: tunic > basal plate > shoot ≥ reserve tissue (Figure 3 b). In contrast, the *Penicillium* distribution gradient decreased as follows: shoot > basal plate ≥ reserve tissue > tunic (Figure 3 b).

For the four clove components (tunic, basal plate, reserve tissue, shoot) of each of the six field clove samples (20 cloves per sample), there was no sample or clove component from which the two fungal genera (*Fusarium* or *Penicillium*) were not co-isolated, with the exceptions of the reserve tissue of clove sample 6 and the shoots of samples 5 and 6, where only *Penicillium* was obtained (Figure S1). *Penicillium* was the prevalent genus isolated from the shoots and reserve tissues in each sample ($P \leq 0.05$), while *Fusarium* was the prevalent genus ($P \leq 0.05$)

Table 2. Symptoms and signs detected on elephant garlic clove components (tunic, basal plate, reserve tissue, shoot), and related incidence per sample. For each symptom type within each clove component, average incidence (± standard error [SE]) was calculated.

TUNIC: symptoms incidence %							BASAL PLATE: symptoms or signs incidence %							
Sample	Pink-purple spotting	MCP[a]	Browning	MCP	Asymptomatic	MCP	Sample	Pink mould	MCP	Grey mould	MCP	Sponge-like rot	MCP	Asymptomatic	MCP
1	35		40		25		1	5		90		0		5	
2	30		70		0		2	0		100		0		0	
3	0		85		15		3	0		20		80		0	
4	95		5		0		4	75		0		5		20	
5	0		10		90		5	0		0		10		90	
6	10		60		30		6	0		0		40		60	
Average	28.3	a	45.0	a	26.7	a	Average	13.3	a	35.0	a	22.5	a	29.2	a
SE	14.6		13.3		13.6		SE	12.4		19.3		13.0		15.3	
RESERVE TISSUE: symptoms incidence %							SHOOT: symptoms or signs incidence %							
Sample	Black-purple streaks	MCP	Rot	MCP	Asymptomatic	MCP	Sample	White mould	MCP	Basal grey mould	MCP	Dry rot	MCP	Asymptomatic	MCP
1	15		85		0		1	0		0		5		95	
2	0		100		0		2	0		60		0		40	
3	0		100		0		3	0		20		0		80	
4	5		95		0		4	10		0		0		90	
5	20		40		40		5	0		10		0		90	
6	0		100		0		6	0		0		10		90	
Average	6.7	a	86.7	b	6.7	a	Average	1.7	a	15.0	a	2.5	a	80.8	b
SE	3.6		9.6		6.7		SE	1.7		9.6		1.7		8.4	

[a]MCP = multiple comparison procedure. Values with different letters are significantly different based on Tukey Honestly Significant difference test ($P < 0.05$).

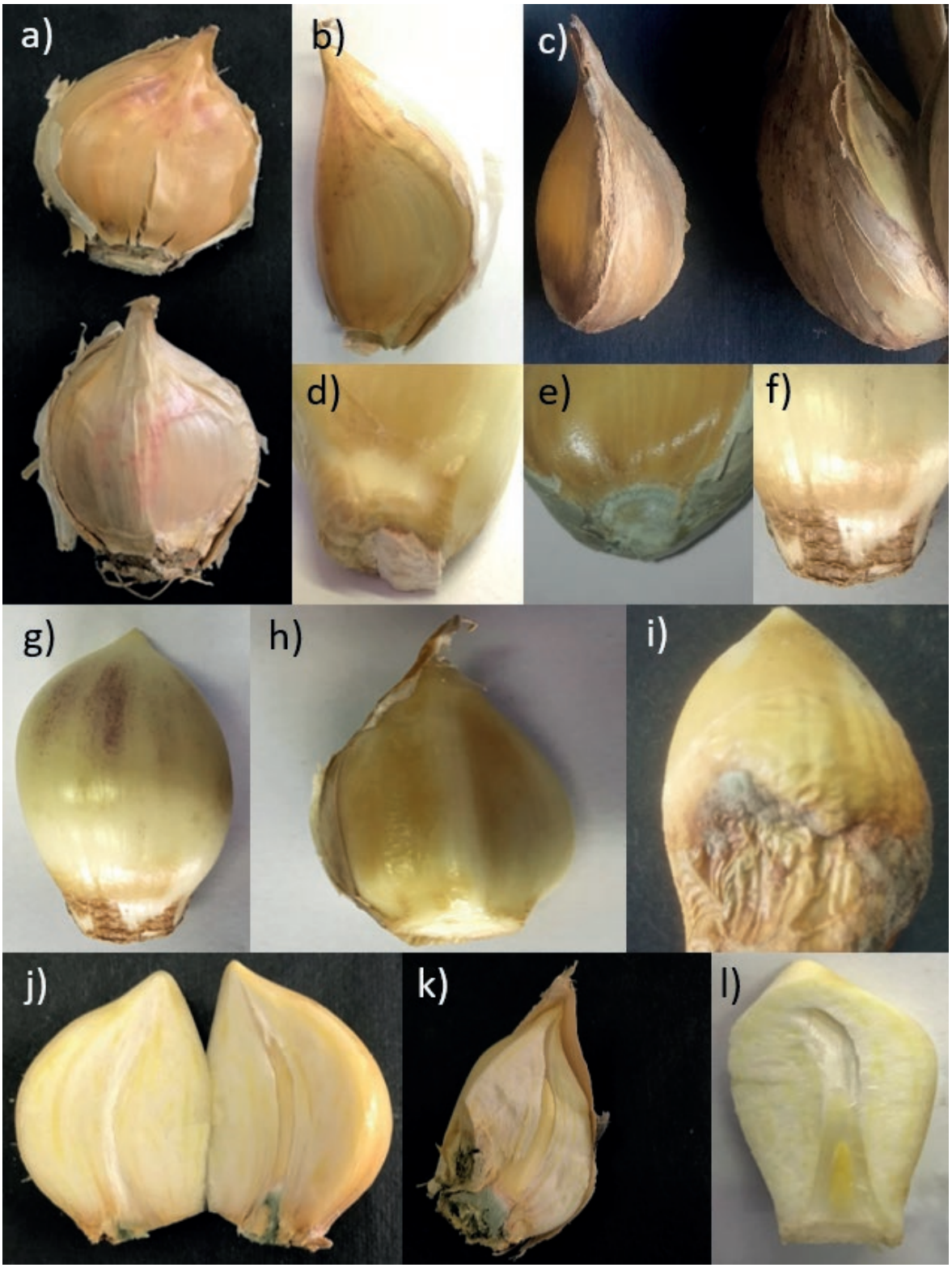


Figure 2. Different symptoms and signs detected on elephant garlic cloves collected in 2019 in "Val di Chiana" area (Central Italy). Pink-purple spotting (a, b) and browning (c) on bulb tunics; pink mould (d), grey mould (e) and sponge-like rot (f) on basal plates; black-purple streaks (g), soft watery rot (h), dry rot and grey mould (i) on reserve tissues; basal grey mould on shoots (j and k), and apparently healthy shoot (I; free of symptoms and signs).

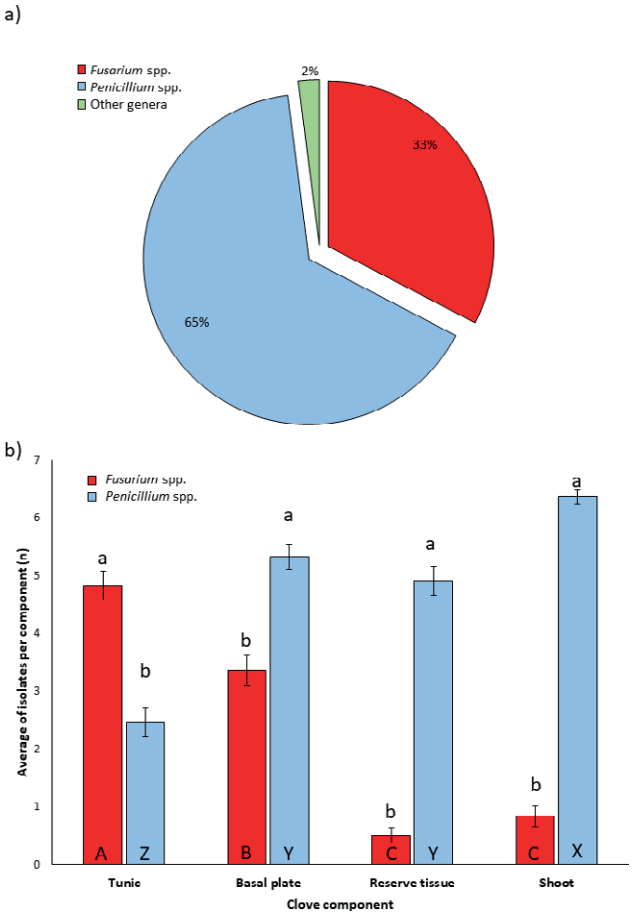


Figure 3. Incidence (%) of isolates of different fungal genera developed from sampled elephant garlic cloves (a), and distribution of the isolates of the two mainly obtained fungal genera (*Fusarium* or *Penicillium*) for each clove component (tunic, basal plate, reserve tissue, shoot) for all sampled cloves (n = 120) (b). Data were subjected to one way analyses of variance (ANOVA), considering fungal genera or clove component as the variables. Columns represent averages (± standard errors) of the number of isolates belonging to each fungal genus. Letters indicate differences ($P \leq 0.05$) between fungal genera within each clove component (letters above error bars) or between clove components within a fungal genus (letters within columns).

isolated from the tunics of three samples and from the basal plates of one sample (Figure S1). Generally, *Fusarium* was isolated less ($P \leq 0.05$) from shoots and reserve tissues of all the field samples compared to tunics and basal plates.

Identification of Fusarium *and* Penicillium *species isolated from elephant garlic cloves*

From the analysed elephant garlic cloves, a total of 47 fungal isolates were collected as representatives of all the observed morphotypes. Of these isolates, 31 were morphologically identified as *Fusarium* spp. and 16 as *Penicillium* spp. This identification was also confirmed by BLAST analysis of the amplified regions (*tef1α* for *Fusarium* spp., *BenA* and *CaM* for *Penicillium* spp.).

Considering the *Fusarium* isolates, according to Crous *et al.* (2021), in the phylogram constructed on the sequences of the *tef1α* gene, two major clades emerged (Figure 4): the first included species of the *Fusarium oxysporum* species complex (FOSC), while the second clade included species of the *Fusarium fujikuroj* species complex (FFSC). In the FOSC clade, three main subclades, here named as 1, 2 and 3, emerged (Figure 4). Subclade 1 included most of the *Fusarium* isolates obtained in this study (16 isolates), which clustered together with reference isolates of *Fusarium oxysporum* f. sp. *cepae* (Table S2). Subclade 2 included only one isolate, which clustered with reference isolates of *Fusarium oxysporum* f. sp. *lactucae*. Subclade 3 included five isolates, which clustered with reference isolates of *Fusarium oxysporum* f. sp. *dianthi*. The FFSC clade included nine isolates which clustered with the reference isolate of *F. proliferatum*.

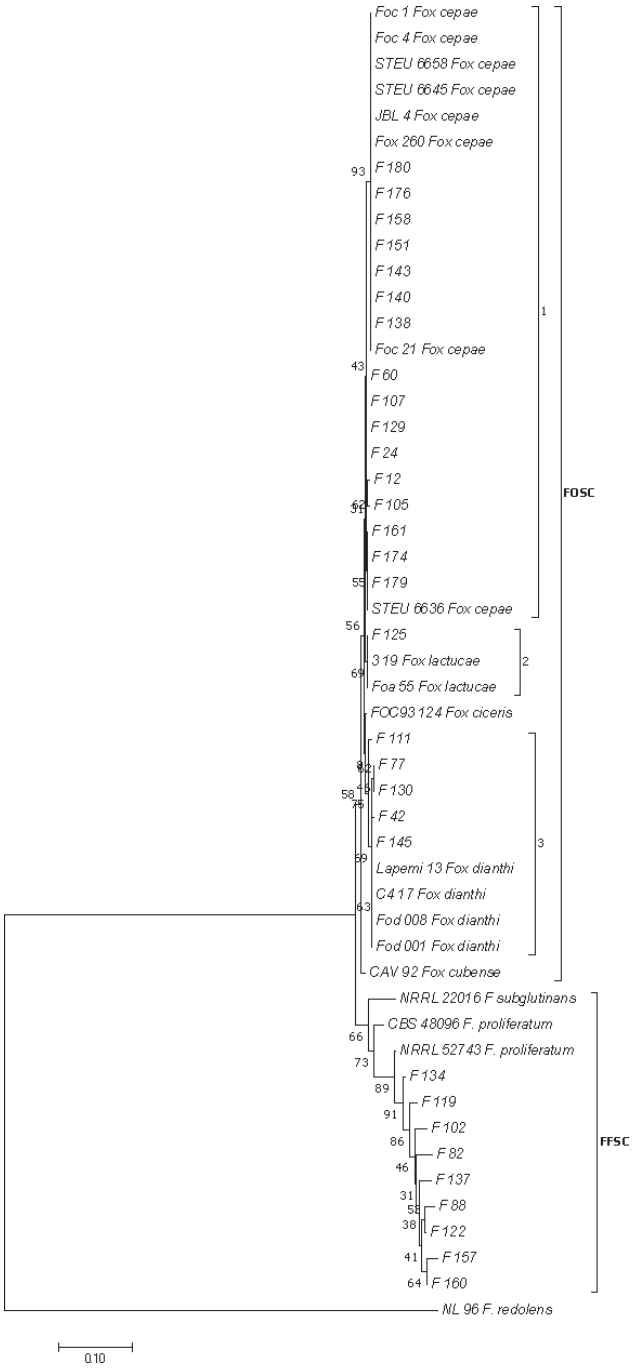
According to Houbraken and Samson (2011) and Visagie *et al.* (2014), in the phylogram constructed on the concatenated sequences of *BenA* and *CaM* genes of *Penicillium* isolates, four major clades emerged (Figure 5). These were: clade A, which included species of the section *Fasciculata*; clade B, which included species of the section *Chrysogena*; clade C, of section *Brevicompacta*; and clade D, of sections *Exilicaulis* and *Citrina*. Most of the isolates (13) clustered in clade A together with a reference strain of *P. allii*. Of the four remaining isolates, two clustered in clade C with reference isolates of *P. brevicompactum*, and one clustered in clade D with reference isolates of *P. citrinum*.

Thus, as indicated by BLAST and phylogenetic analyses, the *Fusarium* and *Penicillium* communities isolated from elephant garlic cloves (tunics, basal plates, reserve tissues, or shoots) were composed of three *Penicillium* species and two different *Fusarium* species (Figures 4, 5 and 6).

The *Fusarium* community (Figures 4 and 6 a) was mainly composed of *F. oxysporum* subclade 1 (56%), followed by *F. oxysporum* subclade 3 (20%), *F. proliferatum* (19%) and *F. oxysporum* subclade 2 (5%). For the different clove components, *F. oxysporum* subclade 1 was the most isolated ($P \leq 0.05$) from bulb tunics and basal plates. *Fusarium oxysporum* subclade 1 was also mainly isolated (but with no statistically significant differences with respect to the other species) from shoots and reserve tissues (Figure 6 a).

Fusarium oxysporum subclade 1 incidence showed differences ($P \leq 0.05$) between the elephant garlic clove

components, as follows: tunics > basal plates > shoots = reserve tissues. A similar trend was also observed for *F. oxysporum* subclade 3 ($P \leq 0.05$: tunics ≥ basal plates ≥ shoots = reserve tissues), and for *F. oxysporum* subclade 2 ($P \leq 0.05$: tunics ≥ basal plates = shoots ≥ reserve tissues). The pattern for *F. proliferatum* was slightly different: tunics = basal plates ≥ shoots ≥ reserve tissues ($P \leq 0.05$).



The *Penicillium* community almost entirely included *P. allii* (95%), which was the most ($P \leq 0.05$) isolated species from all the four clove components. The other two isolated species, *P. citrinum* and *P. brevicompactum*, were less common (4% and 1%, respectively) (Figure 6 b). *Penicillium allii* was the most commonly isolated from all the four clove components, but a pattern ($P \leq 0.05$) was recorded: shoots > basal plates = reserve tissues > tunics. The incidences of isolation from tunics, basal plates, reserve tissues and shoots of the other three species were not significantly different ($P > 0.05$). However, *P. allii, P. brevicompactum* and *P. citrinum* were not different ($P > 0.05$) for the numbers of colonies isolated from the four components of assessed elephant garlic cloves.

The number (n) of *Fusarium* isolates ascribable to the different species/subclades is shown in Figure S2 a. *Fusarium proliferatum*, and *F. oxysporum* subclades 1, 2 and 3 were simultaneously isolated only from the clove tunics of samples 1 and 2, and from the basal portions of sample 2, with no differences ($P > 0.05$) in average incidence. *Fusarium oxysporum* subclade 1 was the only isolated subclade from the basal plates of the six different field samples, but also from all the portions of sample 6. *Fusarium proliferatum* and *F. oxysporum* subclades 1, 2 and 3 did not show differences ($P > 0.05$) in incidence in the reserve tissues and shoots of the six field samples.

For *Penicillium* (Figure S2 b), *P. allii* was the only species isolated from all the examined clove components of the six field samples. In addition, *P. citrinum* was isolated from all the analysed portions of sample 4, and *P. brevicompactum* from the tunics of samples 5 and 6.

Pathogenicity and virulence of Fusarium *and* Penicillium *isolates on elephant garlic cloves*

All *Fusarium* and *Penicillium* isolates used in the pathogenicity tests showed abilities to cause rot symp-

Figure 4. Phylogeny of *Fusarium* isolates obtained in this study. The evolutionary history of *Fusarium* isolates was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) and the dataset of partial *translation elongation factor 1α* (*tef1α*) gene sequences. The optimal tree with the sum of branch length = 1.36523273 is shown. The percentages of replicate trees in which the associated *taxa* clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004), and are in the units of the number of base substitutions per site. The analysis involved 51 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 490 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

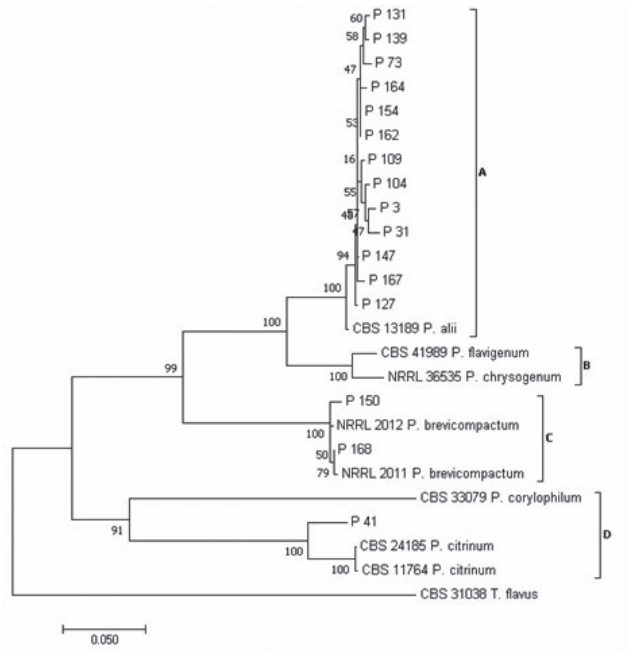


Figure 5. Phylogeny of *Penicillium* isolates obtained in this study. The evolutionary history of *Penicillium* isolates was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) and the combined dataset of partial *β-tubulin* (*BenA*) and *calmodulin* (*CaM*) genes sequencing. The optimal tree with the sum of branch length = 1.04059653 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. The analysis involved 25 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 547 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

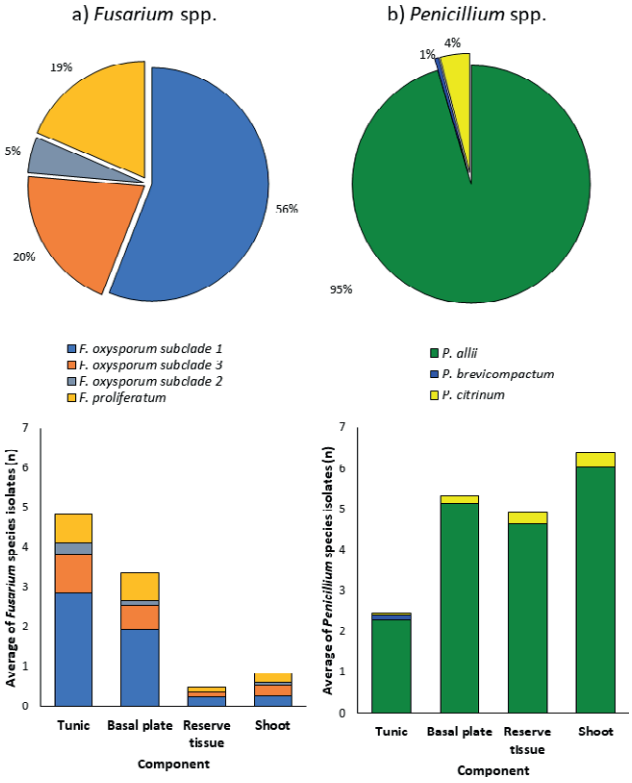


Figure 6. *Fusarium* species/subclades and *Penicillium* species isolated from different elephant garlic clove components from six analyzed field samples. Incidence (%) of the isolated species/subclades, as identified by partial *translation elongation factor 1α* sequencing of *Fusarium* (a), or by *β-tubulin* (*BenA*) and *calmodulin* (*CaM*) partial gene sequencing of *Penicillium* (b), are shown in the pie charts. Columns of the histograms represent the *Fusarium* (a) and *Penicillium* (b) community compositions, expressed as the average number of isolates (n) of different species/subclades that developed on PDA from each analysed elephant garlic clove component.

toms in elephant garlic cloves, so these fungi were shown to be pathogenic towards this crop (Figure 7). However, the tested isolates showed differences in ability to cause clove rots (Figure 7). A gradient in VI was evident for the pathogens (Figure 8), as *P. allii* > *P. brevicompactum* ≥ *F. proliferatum* ≥ *P. citrinum* = *F. oxysporum* subclade 1 ≥ *F. oxysporum* subclade 3 ≥ *F. oxysporum* subclade 2 = experimental control (nil inoculation). The *P. allii* isolate showed greatest virulence ($P \leq 0.05$) compared to the isolates of the other fungi (Figures 7 and 8). *Penicillium brevicompactum* (Figures 7 and 8) and *F. proliferatum* (Figures 7 and 8) were more virulent than *F. oxysporum* subclade 2 ($P \leq 0.05$).

Strategies for the control of Fusarium *and* Penicillium *on elephant garlic cloves*

At 30 days after application, the tested treatments (Table 1) did not affect ($P > 0.05$) garlic clove germination rates compared to the untreated controls (data not shown). No differences ($P > 0.05$) were observed between control or treated cloves for mean shoot length, fresh weight, or dry weight (data not shown). However, the treatments gave different effects on isolation frequency of *Fusarium* and *Penicillium* from the cloves. There were no differences ($P > 0.05$) in frequency of isolations of *Fusarium* spp. from all the clove components between the untreated control, and treatments of Celest Trio®, sodium hypochlorite, sodium hypochlorite + heat treatment, or sodium hypochlorite + heat treatment + Patriot

Figure 7. Symptoms of dry rot on elephant garlic cloves and related sections, at 30 d after fungal inoculations (two mycelial plugs on each clove, taken from 7-d-old cultures grown on potato dextrose agar). Each image is representative of four replicates (cloves). Control (a), *Fusarium proliferatum* (F 88) (b), *F. oxysporum* subclade 1 (F 129) (c), *F. oxysporum* subclade 2 (F 125) (d), *F. oxysporum* subclade 3 (F 42) (e), *Penicillium allii* (P 104) (f), *P. citrinum* (P 41) (g), and *P. brevicompactum* (P 150) (h).

Gold® (Figure 9 a). Only two treatments, Patriot Gold® and Signum®, gave reduced frequency of *Fusarium* spp. isolations ($P \leq 0.05$) compared with the untreated control (Figure 9 a). In comparison to the untreated control, frequency of isolations of *Penicillium* spp. was reduced ($P \leq 0.05$) by all the treatments except sodium hypochlorite (Figure 9 b), with no difference ($P > 0.05$) between the other treatments.

DISCUSSION

The elephant garlic cloves analyzed in this survey showed different symptoms or signs on their four clove components (tunics, basal plates, reserve tissues and shoots). Some of these symptoms or signs have previously been described in elephant garlic cloves (Besoain *et al.* 2002; Ignjatov *et al.* 2019). In addition, some of the symptoms or signs detected in the present study have been previously described on common garlic cloves (Schwartz and Mohan, 2006; Tonti *et al.*, 2012; Gálvez and Palmero, 2021; Horáková *et al.*, 2021; Le *et al.*, 2021; Gálvez and Palmero, 2022). Comparing the symptoms and signs detected in the analyzed cloves with those previously described for elephant and common garlic, there was high probability that the material assessed here was infected by *Fusarium* and *Penicillium* species (Gálvez and Palmero, 2021).

Fusarium and *Penicillium* have also been reported to be associated with asymptomatic cloves of common garlic (Mondani *et al.*, 2021b). Similarly, also in the present

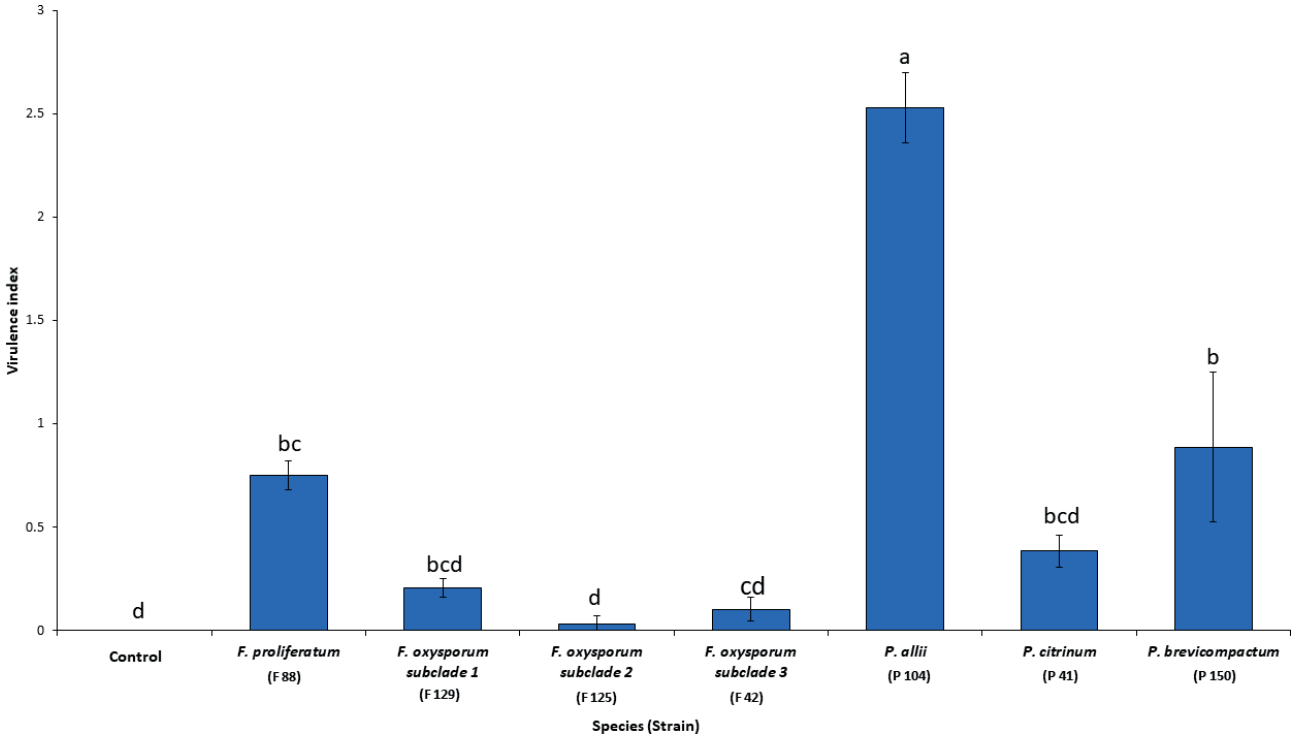


Figure 8. Mean virulence indices for *Fusarium proliferatum* (F 88), *F. oxysporum* subclade 1 (F 129), *F. oxysporum* subclade 2 (F 125), *F. oxysporum* subclade 3 (F 42), *Penicillium allii* (P 104), *P. citrinum* (P 41), and *P. brevicompactum* (P 150), on elephant garlic cloves at 30 days post inoculation. Control cloves were treated with sterile potato dextrose agar plugs. Each column represents the average (± standard error) of four biological replicates, each composed of two cloves with two wounds. Values accompanied by the same letter are not significantly different ($P \leq 0.05$), based on Tukey Honestly Significant Difference multiple comparison tests.

survey, fungi morphologically identified as *Penicillium* and *Fusarium* were isolated both from symptomatic and asymptomatic components of elephant garlic cloves.

Molecular and phylogenetic analyses confirmed the morphological identifications and allowed definition of the fungal species involved.

Penicillium was the prevalent genus, and *Penicillium* isolates were obtained from all the analyzed clove components, with low occurrence in the clove tunics, probably due to the low free water content of these tissues, making them less suitable for *Penicillium* infections (Abellana *et al.*, 2001) than the other three, more hydrated, clove components.

Among the three identified *Penicillium* species, *P. allii* predominated as isolated from all the analyzed elephant garlic clove components collected in "Val di Chiana". The strong predominance of this species was detected also in a previous survey carried out on common garlic in Argentina (Valdez *et al.*, 2009). *Penicillium allii* is the causal agent of green rot or blue mould of common garlic, and has been reported in several countries (Valdez *et al.*, 2006; Dugan, 2007; Moharam *et al.*, 2013; Gálvez and Palmero, 2021). This species is also considered the most aggressive *Penicillium* spp. affecting common garlic in the field and during storage (Overy *et al.*, 2005a; 2005b; Valdez *et al.*, 2009). *Penicillium allii* was also isolated also from soil in Egypt (Vincent and Pitt, 1989).

Penicillium citrinum and *P. brevicompactum* were the other *Penicillium* spp. isolated from cloves analyzed in the present study. *Penicillium citrinum* is of broad geographic distribution, and causes postharvest rots on a wide range of hosts (Wang *et al.*, 2014; González-Estrada *et al.*, 2017; Coutinho *et al.*, 2020; Onaebi *et al.*, 2020; Khan and Javaid, 2023). In addition, *P. citrinum* has been isolated from different substrates including soil, and has been reported as a common endophytic fungus of wheat and soybean (Samson *et al.*, 2004; Khan *et al.*, 2008). *Penicillium brevicompactum* is very widely distributed, especially because of its xerophytic nature (Pitt and Hocking, 1997). This could explain its presence only on the tunics of the elephant garlic cloves analyzed in the present study. This fungus has been reported as a weak pathogen on several fruit types (Overy and Frisvad, 2005), and was isolated from common garlic cloves in Argentina (Valdez *et al.*, 2009).

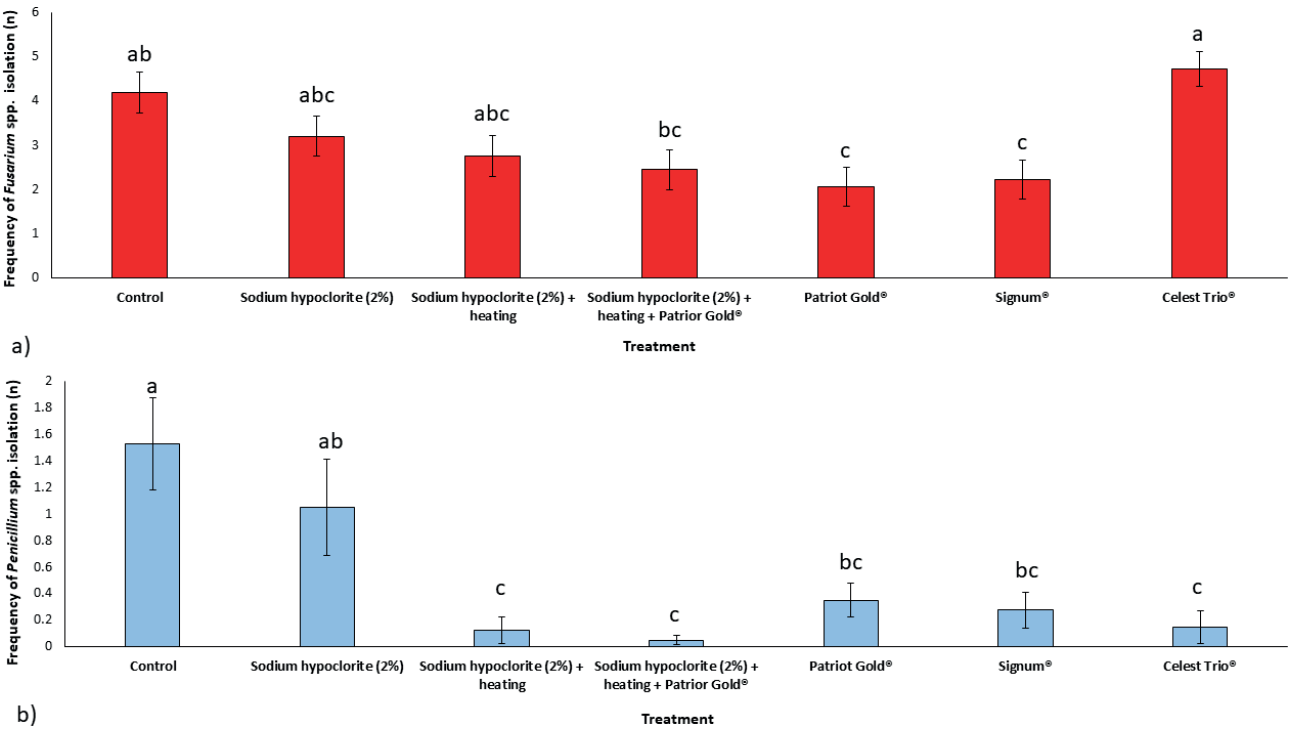


Figure 9. Frequency (n) of *Fusarium* spp. (a) and *Penicillium* spp. (b) isolations from clove components (tunics, basal plates, reserve tissues or shoots) for untreated (Control) or treated naturally infected elephant garlic cloves. Each column represents the average (± standard error) for ten cloves. Different letters indicate differences ($P \leq 0.05$), based on Tukey Honestly Significant Difference multiple comparison tests.

All *Penicillium* species isolated from elephant garlic cloves ready to be used as planting material in "Val di Chiana" have also been isolated from soil (Vincent and Pitt, 1989; Frisvad and Samson, 2004; Samson *et al.*, 2004; Khan *et al.*, 2008; Min *et al.*, 2019). Therefore, *Penicillium* inoculum could infect elephant garlic cloves from the soil, and the pathogens could further develop during storage, particularly when environmental conditions are unsuitable for bulb conservation. *Penicillium allii*, the most isolated species in the present survey, was previously classified as a "field" pathogen, and not as a "storage" pathogen, of common garlic (Valdez *et al.*, 2006). Moreover, *P. citrinum* was isolated only from cloves of a sample obtained from a field where the preceding crop was wheat, which is a commonly reported host for *P. citrinum* (Kaur and Saxena, 2023). These results indicate that the use of infected cloves as planting material and/or preceding crop residues provide or increase *Penicillium* inoculum in soil. The results also underline the importance of crop rotations that avoid cultivation of *Allium ampleoprasum* var. *holmense* after other *Allium* spp., as well as well-proven hosts of these pathogens.

Penicillium spp. were more frequently isolated than *Fusarium* spp. from elephant garlic clove components. *Fusarium* infections may have resulted from soil inoculum, this fungal genus was isolated more frequently from clove components (tunics and basal plates) in contact with the soil. The basal plates were indicated, together with roots, as the most important infection sites of *Fusarium* spp. causing *Fusarium* basal rot (FBR) (also known as *Fusarium* dry rot) on common garlic cloves (Le *et al.*, 2021). This is because FBR is also a "soil-borne" disease, and the causal agents can survive as chlamydospores or as saprophytes in crop residues (Le *et al.*, 2021). However, FBR is not only a "soil-borne" disease and the soil may not be the major reservoir of inoculum, because, for example, *F. proliferatum*, one of the FBR causal agents, does not form chlamydospores (Elmer *et al.*, 1999). *Fusarium* inoculum, from soil or from planting material, is important for causing field infections, while latently infected garlic cloves mainly contribute to post-harvest rots of *Allium* spp. (Stankovic *et al.*, 2007; Gálvez *et al.*, 2017a; Le *et al.*, 2021;), also compromising quality of planting material. This can become an additional source of inoculum in the field (Le *et al.*, 2021).

Fusarium oxysporum was the most isolated *Fusarium* species, followed by *F. proliferatum*. As reported in common garlic (Stankovic *et al.*, 2007; Mondani *et al.*, 2021a), these two *Fusarium* spp. frequently co-occur in

garlic cloves (Le *et al*., 2021; Gálvez and Palmero, 2022).

Fusarium proliferatum is a well-known FBR pathogen of common garlic and onion crops (Mahmoody, 1998; Dugan *et al*., 2003; Stankovic *et al*., 2007; Palmero *et al*., 2010; Sankar and Prasad Babu, 2012; Tonti *et al*., 2012; Fuentes *et al*., 2013; Salvalaggio and Ridao, 2013; Ignjatov *et al*., 2017; Leyronas *et al*., 2018). In most cases, *F. proliferatum* has been the predominant *Fusarium* species associated with FBR in common garlic (Chrétien *et al*., 2021; Gálvez and Palmero, 2022). In addition, *F. proliferatum* has also been reported from elephant garlic in Serbia (Ignjatov *et al*. 2019). Due to its polyphagous behaviour, *F. proliferatum* has been reported as a cosmopolitan pathogen of many important crop plants (Gálvez and Palmero, 2022). For this reason, inoculum of this fungus is likely to be present in field soils. Some hosts (maize, wheat, potato, and sunflower) can serve as inoculum sources (Molinero-Ruiz *et al*., 2011), and severe *F. proliferatum* infections can be expected in susceptible crops such as garlic types. These aspects should be considered by elephant garlic growers, to define the rotations within their cropping systems. Assessment would be worthwhile of the ability of *F. proliferatum* strains detected from elephant garlic to cause disease in other hosts. *Fusarium proliferatum* can also biosynthesize mycotoxins, such as fumonisins, that can induce toxic effects in humans (Kamle *et al*., 2019). Elephant garlic cloves, even though in small quantities, are used for human consumption, so it is important that accumulation of mycotoxins is monitored, as has already been investigated in common garlic (Tonti *et al*., 2017).

Fusarium oxysporum was also isolated in the present study with high incidence from elephant garlic cloves. This fungus has already been reported in elephant garlic in Chile (Besoain *et al*., 2002). Identifications based on *tef1α* showed that *F. oxysporum* isolates obtained in the present study were in three subclades, most closely associated with f. sp. *cepae*, f. sp. *dianthi* or f. sp. *lactucae*. However, further study is required, particularly of isolate gene sequences, to ascribe these isolates to different *formae speciales* (El-Komy *et al*., 2023). *Fusarium oxysporum* is a soil-borne pathogen, well-known as an FBR agent in different *Allium* spp. including onion, common garlic and shallot (Schwartz and Mohan, 2006; Sintayehu *et al*., 2011; Mondani *et al*., 2021a; Gálvez and Palmero, 2022). Mondani *et al*. (2021a) conducted a survey of common garlic basal plates from bulbs cultivated in Northern Italy, and showed that *F. oxysporum* was favoured by dry weather, differently from *F. proliferatum* which was favoured by rain.

Representative isolates of each *Penicillium* and *Fusarium* species were shown to be pathogenic on elephant garlic cloves. The inoculation technique adopted, as described elsewhere (Dugan *et al*., 2007; Ignjatov *et al*., 2019), is invasive to host tissues, so did not allow verification of relationships between the pathogen species and associated host symptoms. For this purpose, further study is required, including inoculating spore suspensions directly onto cloves or into soil.

In the experimental conditions used in the present study, *P. allii* was the most virulent fungus followed by *P. brevicompactum*, *F. proliferatum* and *F. oxysporum*. The greater virulence of *P. allii* in comparison to *Fusarium* spp. (Gálvez and Palmero, 2021), or other *Penicillium* spp. (Overy *et al*. 2005a and 2005b; Valdez *et al*. 2009) has been previously reported for common garlic. Also in elephant garlic cloves, *P. hirstum* had greater virulence than *F. oxysporum* (Besoain *et al*., 2002). The greater aggressiveness of *P. allii* could be attributed, as already suggested by Valdez *et al*. (2009), to the ability of this fungus to utilize enzymatic digestion of host cell wall polymers.

Appropriate disease management strategies are required for elephant garlic, but no management protocols are currently available for this "niche" crop in Italy, which is cultivated in a limited area. Farmers adapt to elephant garlic control methods for similar crops, such as common garlic, onion, or leek. Since fungal pathogens can reach the field also through infected garlic cloves used as planting material, a first step to reduce pathogen inoculum added to soil from infected planting material could be clove disinfection. The present study tested different disinfection treatments. Among these, the products Signum® and Patriot Gold® showed efficacy for control of *Penicillium* spp. and *Fusarium* spp. Instead, Celest Trio® and sodium hypochlorite (2%), combined with heat treatments, or with heating and Patriot Gold®, reduced *Penicillium* spp. only.

Several studies have assessed effectiveness of chemical treatments for reducing *Fusarium* occurrence in crops similar to elephant garlic, including common garlic. These studies demonstrated effectiveness of several fungicide active ingredients, including benomyl (Dugan *et al*., 2007), carbendazim, metalaxyl + mancozeb, tiophanate-methyl (Elshahawy *et al*., 2017), propiconazole + prochloraz, or tebuconazole (Mondani *et al*., 2021c; 2022), for reducing *F. proliferatum* and *F. oxysporum* growth *in vitro* and/or isolation frequencies *in vivo*. However, authorization for the use of some of these active ingredients (benomyl, carbendazim, propiconazole and prochloraz) has been recently revoked by the European Commission. Interest in biofungicides in crop protection has rapidly increased (Chandler *et al*., 2011). For example, *Bacillus subtilis* and *Streptomyces*

griseoviridis showed the promise for control of *F. proliferatum* comparable to that from chemicals on common garlic cloves. In contrast, *Trichoderma harzianum* + *T. gamsii*, which showed potential for reducing *in vitro* growth of *F. proliferatum* and *F. oxysporum*, was not fully confirmed for reducing disease *in vivo* (Mondani *et al.*, 2021c). However, *B. subtilis* or *T. harzianum* + *T. gamsii* based products have yet to be authorized for use on common garlic, onion, or leek in Italy, and therefore their use cannot be extended to elephant garlic. The heat treatment in the experimental conditions used in the present study did not negatively affect plant emergence from treated garlic cloves. Application of thermotherapy (using hot water) could be deleterious to emergence, if combinations of temperature and time are not carefully assessed. When this technique was assessed for common garlic, its practical application was considered to be difficult, and effectiveness could decrease if pathogen mycelium had entered host clove lesions (Palmero Llamas *et al.*, 2013). The present study recorded efficient control of *Fusarium* spp. and *Penicillium* spp. with Patriot Gold® and Signum® (both currently approved in Italy for use on common garlic, onion, or leek, and therefore potentially extendable for use on elephant garlic). Therefore, these treatments are likely to be for managing these increasingly threatening pathogens of elephant garlic cloves.

In conclusion, the present study was the first to focus on phytosanitary problems of elephant garlic cultivated in Italy, and was a preliminary assessment of possible disease management solutions. Choice of healthy, symptom/sign-free cloves remains the first step for producing healthy crops. However, since *Fusarium* and *Penicillium* were also isolated from symptom/sign-free garlic cloves, application of disinfection methods is the second, and necessary, step as common practice for elephant garlic cultivation. The preliminary, positive disease control results obtained should be further investigated and confirmed. The several current and registered active ingredients (both chemical and microbiological) could be accompanied or replaced by new solutions, making an evolving phytoiatric scenario for elephant garlic production.

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