Occurrence of the Hyperparasite *Ampelomyces quisqualis* on *Golovinomyces neosalviae* (Erysiphaceae), Causal Agent of Powdery Mildew on Common Sage (*Salvia officinalis*)

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Abstract

Hamzeh, S., W. Naffaa and M.F. Azmeh. 2022. Occurrence of the Hyperparasite *Ampelomyces quisqualis* on *Golovinomyces neosalviae* (Erysiphaceae), Causal Agent of Powdery Mildew on Common Sage (*Salvia officinalis*). Arab Journal of Plant Protection, 40(2): 158-163. https://doi.org/10.22268/AJPP-040.2.158163

Ampelomyces quisqualis, the oldest mycoparasite of powdery mildew (PM), has been widely studied due to its potential in biocontrol. Many strains of this hyperparasite have been experimented worldwide and some of them were successfully applied for biocontrol, but others have been less efficient. No previous identification of *Ampelomyces* strains has been done in Syria, but some isolates were morphologically identified in the coastal region. There was no indication of *Ampelomyces* occurrence in any other location in Syria. During this three years survey (2019–2021), 73 plant samples were collected from five governorates, including coastal and southern regions in Syria. *Ampelomyces* pycnidia were detected in five samples from the coastal area and a new unpredictable finding of *Ampelomyces* sp. was found in southern Syria. This new occurrence of *Ampelomyces* isolate (S.ham82) is documented on a new mycohost; *Golovinomyces neosalviae*, the causal agent of PM on common sage (*Salvia officinalis*). Successful isolation of S.ham82 on PDA was conducted and parasitic activity was assessed by artificial inoculation using *In vitro* detached leaf assay. Morphological characteristics of this isolate were determined and compared with isolate Bah1 from the coastal region. *Ampelomyces* sp. (S.ham82) pycnidia size were 77.44 (±17.16) x 25.28 (±6.12) µm in natural host, 125.27 (±42.34) x 115.95 (±40.14) µm, 189.51 (±60.06) x 167.64 (±52.41) µm on PDA media pre and post artificial inoculation, respectively, and 88.24 (±20.05) x 27.98 (±5.68) µm on inoculated detached leaves. Conidia were also morphologically characterized and measured 8.11 (±0.87) x 3.88 (±0.51) µm in natural host, 8.86 (±1.65) x 3.18 (±0.80) µm in PDA pre and post artificial inoculation, respectively, and 7.82 (±0.69) x 3.61 (±0.37) µm in inoculated detached leaves. To our knowledge, this is the first report of the natural occurrence of *Ampelomyces* sp. in *G. neosalviae* on *Saliva officinalis*.

Keywords: *Ampelomyces* sp., Common sage, *Golovinomyces neosalviae*, Syria.

Introduction

The most widespread and oldest known natural enemies of powdery mildew (PM) *Ampelomyces* spp. are intracellular mycoparasites where its hyphae grow inside the mycelia of their hosts killing the PM hyphae by degeneration of the cell content (Falk *et al*., 1995a, 1995b; Hashioka & Nakai, 1980; Kiss, 2008; Kiss *et al*., 2004). The early stages of hyperparasitism are seemingly obligate, and death of invasive PM colonies is initiated by direct consumption mechanism of host cell bioenergy (Hashioka & Nakai, 1980; Sandheim & Krekling, 1982). *Ampelomyces* can spread to long distances as hyphal fragments in parasitized and detached PM conidia (Jarvis & Slingsby, 1977). When these parasitized air-borne conidia within or in proximity of any PM colony under humid conditions, the outgrowing hyphae of *Ampelomyces* can penetrate their mycelia (Kiss *et al*., 2004). As pesticide, *A. quisqualis* is the active ingredient of the oldest mildew bio-fungicide commercially known as AQ10™ and other products based on different *Ampelomyces* strains such as Q-fect WP developed in Korea (Lee *et al*., 2004). The natural occurrence of *A. quisqualis* on various Erysiphaceae species has been reported in different geographic regions worldwide (Angeli *et al*., 2009; Kiss, 1997; Kiss *et al*., 2004; Rankovic, 1997). Kiss (1998) recorded the occurrence of *Ampelomyces* spp. in 570 samples representing 27 species (nine genera) of the Erysiphaceae infecting 41 host plant genera. In Syria, the coastal area was the only known habitat of *A. quisqualis* on PMs according to the study published by Younes *et al*., (2009), indicating the occurrence of *Ampelomyces* on 29 species of PM belonging to 8 different genera on 59 plant species distributed in 22 families. Therefore, no previous studies have mentioned the occurrence of *Ampelomyces* in any other location in Syria. Furthermore, none of the previous studies mentioned the occurrence of the hyperparasite on *Golovinomyces neosalviae* (*Erysiphe salivae* Blumer), parasiting *Salvia officinalis*, previously recorded by Cabrera *et al.* (2010) and Götz *et al.* (2018). To our best knowledge, *Ampelomyces* parasitism on *G. neosalviae* has not been previously reported in Syria or elsewhere. Many factors related to the appropriate environmental conditions control the occurrence of *Ampelomyces* on PM such as temperature, humidity and other factors. Most of previous investigations have emphasized the great need to high relative humidity (RH) for internal growth and sporulation of *Ampelomyces* strains (Jarvis & Slingsby, 1977; Philipp & Crüger, 1979). Therefore, there is interest to discover the occurrence *Ampelomyces* isolates in new locations in Syria and investigate further this pycnidial parasite of PM fungi and how could we benefit from it.

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This study focused on proving and documenting the occurrence of *A. quisqualis* in a new PM mycohost, *G. neosalviae* on common sage and in a new ecologically different area in Syria, as well to confirm the occurrence of *A. quisqualis* in the coastal area.

Materials and methods

Field surveillance

Several surveys per year were conducted during 2019, 2020 and 2021 to detect the presence of *Ampelomyces* mycoparasites in PM colonies growing on different host plants. Plant samples infected by their respective mildew pathogens were collected from 10 locations distributed in three governorates (Damascus, Damascus countryside and Sweida) in southern Syria as well as from 20 locations distributed in two coastal governorates (Lattakia and Tartous) in Syrian. Samples were transferred to the laboratory and examined microscopically for the possible presence of *Ampelomyces* sp. (Table 1).

Isolation and morphological characterization of *Ampelomyces* sp. (S.ham82)

The presence of *Ampelomyces* pycnidia was examined using stereomicroscope (optic ivymen system). For confirmation, the observed pycnidia were re-examined again using light microscope (BIOBASE) by applying a slight pressure on the slide-cover to force pycnidium wall to rupture releasing conidia. Pycnidia were transferred to potato dextrose agar medium (PDA) using sterile hand-made glass needles (Goh, 1999), incubated at 25°C, to obtain pure cultures. Morphological characters of *Ampelomyces* sp. (S.ham82) such as hyphae, pycnidia and conidiogenous cells were examined using stereo and light microscope. In addition, Daily diameter measurement was performed to determine the growth rate of *Ampelomyces* colonies. Morphological characters of hyperparasite structures were determined in natural host and on PDA medium.

For artificial inoculation, a conidiospore suspension was made by transferring several pycnidia from pure culture to 1ml tube filled with 500 µl sterile distilled water and 0.1% Tween 80, mixing with stirrer for a minute and waiting for few hours to allow spores to liberate through ruptured pycnidia walls. Concentration of spore suspension was adjusted using Neubauer chamber.

Mycoparasitism activity of *Ampelomyces quisqualis* (S.ham82 isolate) against common sage PM under controlled conditions

Detached leaf assay was performed as described by Zang *et al*. (2020) with some modifications. Leaves of common sage (*Salvia officinalis*) were collected from previously cultivated plants in pots for *in vitro* artificial inoculation. A pair of 9 cm petri dishes were mounted (one above the other) as explained in Figure 1. The upper plate was stuck to the lid of the bottom one and a hole was made in the stuck dish layers to allow passage of the leaf petiole. Detached leaf was put in the upper plate and its petiole was allowed to penetrate the hole towards the down petri dish filled with sterile water for leaf survival and as moist source.

Table1. Plant hosts infected with PM collected during seasons 2019, 2020 and 2021 in southern and coastal regions of Syria.

Location	Plant hosts	PM species
SOUTHERN AREA		
Damascus		
Abo Jarash	*Euonymus japonicus*	*Erysiphe euonymi-japonici*
	Convolvulus sp.	*Erysiphe convolvuli*
	Conyza bonariensis	*Podosphaera erigerontis*
	Salvia officinalis	*Golovinomyces neosalviae**
Fac. Of Mech. & Elect Eng	*E. japonicus*	*E. euonymi-japonici*
Damascus	*C. arvensis*	*E. convolvuli*
Damascus Countryside		
Sasaa	*C. arvensis*	*E. convolvuli*
	Helianthus annuus	*Sphaerotheca fuliginea, Oidium (Blumer)*
Ghouta	*Polygonium* sp.	*E. polygonii*
Jaramana	*C. arvensis*	*E. convolvuli*
	Cucurbita maxima	*S. fuliginea, E. cichoracearum*
	Cucurbita pepo	*S. fuliginea, E. cichoracearum*
Nabk	*C. arvensis*	*E. convolvuli*
Sweida		
Orman mountin	*C. arvensis*	*E. convolvuli*
Qanawat	*Vitis vinifera*	*Uncinula necator*
Reemt Allohf	*V. vinifera*	*U. necator*
COASTAL AREA		
Tartous		
Akkar	*Cucurbita maxima*	*P. xanthii*
Alkhrab	*C. arvensis*	*E. convolvuli*
Banias	*Solanum-lycopersicum*	*Leveillula- taurica*
	S. melongena	*L. taurica*
Hrysoun	*S. lycopersicum*	*L. taurica*
	V. vinifera	*U. necator*
Lattakia		
Lattakia	*C. bonariensis*	*P. erigerontis*
Kangrah	*C. arvensis*	*E. convolvuli*
Krrameh,Bahl-ouliyah	*C. arvensis*	*E. convulvuli*
	Hibiscus esculentus	*E. cichoracearum*
	Trifolium sp.	*Microsphaera trifolii*
Bahlouliyah	*C. arvensis*	*E. convolvuli*
	H. esculentus	*E. cichoracearum*
	Ammi majus	*E. heraclei*
Qasmin	*C. arvensis*	*E. convolvuli*
	Cucurbita pepo	*P. xanthii*
	H. esculentus	*E. cichoracearum*
Knysat	*Capsicum* sp.	*L. taurica*
	Inula sp.	
Shamiyah	*H. esculentus*	*E.cichoracearum*
Amrouniyah	*C. arvensis*	*E. convolvuli*
	H. esculentus	*E. cichoracearum*
Burj Eslam	*Quercus* sp.	*Microsphaera alphitoides*
Daatoor	*C. arvensis*	*E. convolvuli*
Mashkita	*Melia azedarach*	*Phyllactinia guttata*
Demsarkho	*V. vinifera*	*U. necator*
	Urospermum sp.	*E. cichoracearum*
Hmeimim-Gableh	*Cucumis sativus*	*E. cichoracearum, S. fuliginea*
	Ainsworthia sp.	*E. heraclei*
Snoubr	*Solanum sp.*	*L. taurica*
Hennadi	*Ammi majus*	*L. umbelliferarum*
Ras Shumra	*C. arvensis*	*E. convolvuli*

*new record

Spore suspension ($4.4x10^6$ conidia/ml) of S.ham82 was prepared as described above, and 20 µl were sprayed on leaves surfaces, then fresh mildew spores collected from infected leaves of common sage were immediately spread on the leaves surfaces previously inoculated with *Ampelomyces* using a soft brush. Inoculated petri dishes were sealed, incubated at 25°C for 24 h in dark, and then transferred to 12/12 light and dark system (Zang *et al*., 2020).

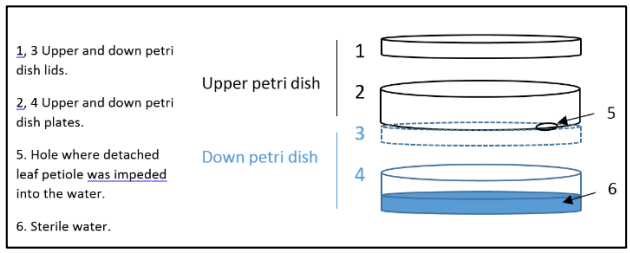

Figure 1. Manipulation of petri dishes for the detached leaf assay

Results and discussion

Field surveillance

Seventy-three wild and cultivated plant samples infected with PM were collected during the seasons 2019, 2020 and 2021. *Ampelomyces quisqualis* was detected in six specimens belonging to three different genera of PM on three host plants. *A. quisqualis* was detected in four plant samples of *Convolvulus* sp. infected with *Erysiphe convolvuli* and collected from four locations (Qasimin, Krrameh, Bahlouliayh and Amrouniyah), and one plant sample of *Ammi majus* infected with *E. heraclei* and collected from Bahlouliyah along the Syrian coast during 2019 and 2020. A new occurrence of *A. quisqualis* was observed on common sage (*S. officinalis*) parasitized by *G. neosalviae* PM which is not included in mildew species listed by Younes *et al.* (2009) in Syria, nor in previous international studies (Kiss, 1998, 2003; Liang *et al.*, 2007). Moreover, our observation of *A. quisqualis* during the 2021 season was detected in Damascus, where its occurrence was not expectable. In general, southern Syria environment is inappropriate for *Ampelomyces* fungus, due to low levels of RH most of the year with some exceptions, so it was a surprise to find this mycoparasite in such region. This finding gave a motivation to confirm the presence of this isolate and later to test it as a candidate for biological control against PM diseases.

Morphological characteristics of *Ampelomyces* S.ham82 isolate

All *Ampelomyces* isolates diagnosed in this study were capable to form pycnidia 5-7 days post inoculation (dpi) and this was in agreement with Kiss (2008), and developed mature ones 8-10 dpi.

Morphological characteristics of *Ampelomyces quisqualis*

In natural infections, pycnidia shape varied from fusiform to ovoid, pedicellate, light to dark brown in color and measured 45.72 - 107.5 x 18.29 - 46.23 µm [av. (±SD) 77.44 (±17.16) x 25.28 (±6.12), n = 20]. Conidia were unicellular, hyaline, discrete, varying in shape from ovoid, pyriform, oblong, globose and measuring 6.25 - 10 x 3 – 5 µm [av. (±SD) 8.11 (±0.87) x 3.88 (±0.51), n = 20] (Figure 2-b and 2-d).

On PDA culture medium, pycnidia were mostly globose to sub-globose and measured 50.29 - 205.74 × 45.72 - 205.74 µm [av. (±SD) 125.27 (±42.34) x 115.95 (±40.14), n = 25]. Conidia were unicellular, hyaline, discrete, varying in shape from ovoid, flask shaped, oblong, curved, globose and measuring 5 - 12.5 x 2.5 – 5 µm [av. (±SD) 8.86 (±1.65) x 3.18 (±0.80), n = 25] (Figure 2-e).

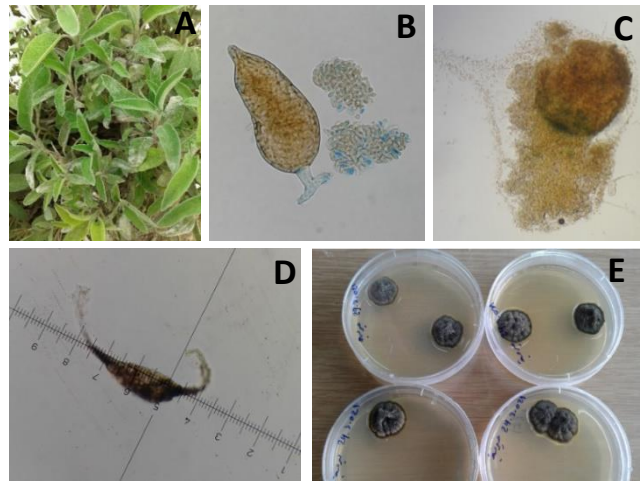

Figure 2. Occurrence of *Ampelomyces quisqualis* (S.ham82) on PM *Golovinomyces neosalviae* infecting common sage (*Salvia officinalis*). A) Common sage infected with Powdery mildew. B) *A. quisqualis* pycnidium isolated from powdery mildew infected common sage. C) Pycnidium with released conidia of cultured mycoparasite on PDA. D) Pycnidia measurement of *A. quisqualis*. E) Colonies of hyperparasite on PDA.

On detached leaf after artificial inoculation, pycnidia were mostly fusiform and measured 45.72-137.16 x 18.288-41.15 µm [av. (±SD) 88.24 (±20.05) x 27.98 (±5.68), n = 25]. Conidia were unicellular, hyaline, discrete, varying in shape from ovoid, pyriform, oblong, globose and measuring 4.5-8.75 x 2.5-4.5 µm [av. (±SD) 7.82 (±0.69) x 3.61 (±0.37), n = 14].

On PDA culture medium following artificial inoculation, pycnidia were globose to sub-globose and measured 114.3-293.8 × 91.44-262.89 µm [av. (±SD) 189.51 (±60.06) x 167.64 (±52.41), n = 15]. Conidia were unicellular, hyaline, discrete, varying in shape from ovoid, flask shaped, oblong, curved, globose, and measuring 5 - 12.5 × 2.5 – 5 µm [av. (±SD) 8.86 (±1.65) x 3.18 (±0.80), n = 15].

Isolate S.ham82 was compared morphologically on PDA with Bah1 (an *A. quisqualis* isolate from coastal area in this study). Several characteristics studied earlier by Sharma (2006) were used in this comparison, in addition to pycnidia and conidia dimensions (Table 2). In our study, pycnidia and conidia of S.ham82 measured 45.72-107.5 x 18.29-46.23 µm

and 6.25-10 x 3–5 µm respectively in natural mycohost which are nearly identical to those obtained earlier (45 – 106 x 25.5–40.5 µm and 4.5–10.5 x 2.5–4.8 µm), respectively (Rancovic, 1997). Younes *et al*. (2008) documented the size of pycnidia and conidia on PDA as follows: 118.387(±26.676) x 113.575(±26.565) µm and 9.433(±1.804) x 3.561(±0.465) µm, respectively, whereas S.ham82 pycnidia and conidia measured slightly larger (125.27 (±42.34) x 115.95 (±40.14) µm and 8.86 (±1.65) x 3.18 (±0.80) µm, respectively, on PDA. Athira *et al*. (2017) reported the radial and fluffy raised growth pattern of different isolates of *Ampelomyces*. Pycnidia were ovoid, ellipsoid, cylindrical, pyriform to globose in shape, measuring 29.2 - 72.5 x 22.4 - 43.1 µm.

All isolates studied in this work were classified as slow growing isolates, S.ham82 and Bah1 isolates had radial growth rates of 0.54 and 0.37 mm day^{-1}, respectively. This result is in accordance with earlier work (Mhaskar & Rao, 1974; Rudakov, 1979; Kiss & Vajna, 1995; Kiss, 1997; Kiss & Nakasone, 1998) who distinguished between slow isolates with a radial growth rate of 0.1-1mm day^{-1}, and fast ones characterized by 3–4mm day^{-1} radial growth in culture media at room temperature.

Data in Table 2 show that S.ham82 and Bah1 isolates were similar in some characters and different in others. Concerning the pycnidia shape, both were globose to sub-globose on PDA medium but Bah1 pycnidia were slightly larger (152.52 x 132.13 µm) compared to S.ham82 pycnidia (125.27 x 115.95 µm). Margins, zonation and topography characteristics of their colonies were similar. On the other hand, S.ham82 had a slightly faster growth rate (0.54 $mm.day^{-1}$) compared with Bah1 isolate which had a slower growth rate (0.37 $mm.day^{-1}$). S.ham82 colonies had dark brown to black color, whereas Bah1 colonies were greenish to brown. Lemon shaped conidia were observed only in Bah1 isolate and conidia size was 8.75 x 3.30 µm. Other conidial characteristics were similar in shape and size with S.ham82 isolate which had conidial size of 8.11 x 3.88 µm.

Mycoparasitism activity of S.ham82 isolate against common sage PM in detached Leaf

Artificial inoculation produced a successful growth of the hyperparasite on PM colonies grown after artificial inoculation. *Ampylomyces* spread inside the mycelia of mycohost and produced pycnidia on most of mildew colonies. Moreover, re-isolation of the mycoparasites was repeatedly successful from the inoculated PM colonies. Based on Koch's postulate, *A. quisqualis* (S.ham82) isolate was found to be a new pathotype which parasitize *G. neosalviae* the causal agent of powdery mildew on common sage (*S. officinalis*) (Figure 3). In addition, S.ham82 could be a tolerant mutant of hyperparasite, having the ability to survive in low RH environment, while *Ampelomyces* need high levels of humidity for conidiospores germination and growth (Jarvis & Slingsby, 1977; Philipp & Crüger, 1979).

It can be concluded that *A. quisqualis*, the oldest parasite of PM fungi, has the potential to colonize the pathogen *G. neosalviae*, the causal agent of mildew on common sage (*S. officinalis*). It was also observed that this hyperparasite may grow in dryer ecosystem than what was reported earlier in Syria. Such isolate should attract more attention and investigated further for its use as a biocontrol agent. Molecular characterization should be performed on S.ham82 isolate to confirm whether or not it is a new strain of *A. quisoualis* parasitic on *G. neosalviae.*

Table 2. Brief comparison of S.ham82 isolate with Bah1 isolate obtained from the coastal area during the 2019 season grown on PDA culture medium.

Characteristics	S.ham82	Bah1
Pycnidia shape	mostly globose to sub-globose	mostly globose to sub-globose
Size	125.27 x 115.95 µm	152.52 x 132.13 µm
Conidia shape	unicellular, hyaline, discrete, ovoid, flask shaped, oblong, curved, globose	unicellular, hyaline, discrete, lemon and flask shaped, oblong, curved, globose
Size	8.11 x 3.88 µm	8.75 x 3.3 µm
Colony radial growth rate/day	0.54mm	0.37mm
Color	Dark brown to black	Brown to greenish brown
Margin	Wavy to diffuse	Wavy to diffuse
Zonation	Present/radial sectors	Present/radial sectors
topography	Septate, Hyaline	Septate, Hyaline
(Mycelium growth nature)	Fluffy when young then tufty	Fluffy when young then tufty

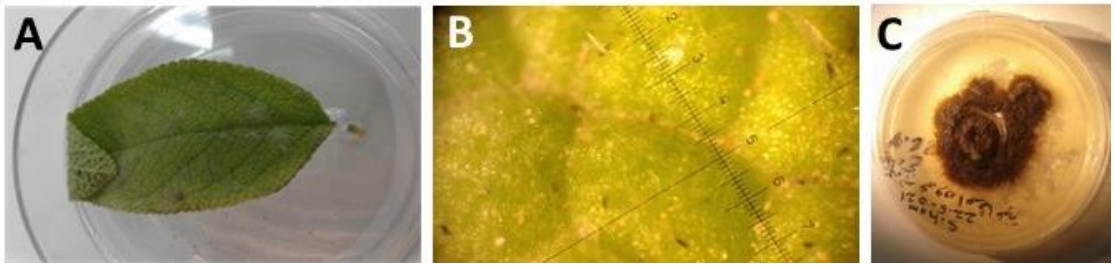

Figure 3. Detached leaf assay. a) Detached leaf of *S. officinalis* inoculated with *G. neosalviae* and bearing pycnidia of mycoparasite. b) Pycnidia spread on detached leaf after inoculation. c) Re-isolation on PDA and successful growth of the mycoparasite.

الملخص

حمزة، شادي، وليد نفاع ومحمد فواز العظمة. 2022. ظهور الفطر فائق التطفل *Ampelomyces quisqualis* على الفطر *Golovinomyces neosalviae* (Erysiphaceae) المسبب لمرض البياض الدقيقي على المريمية الشائعة *Salvia officinalis*. مجلة وقاية النبات العربية، 40(2): 158-163. https://doi.org/10.22268/AJPP-040.2.158163

تمّت دراسة الفطر *Ampelomyces quisqualis*، الذي يعدّ من أقدم الفطور المتطفلة على فطور البياض الدقيقي، على نطاق واسع نظراً لإمكانية استخدامه في المكافحة الحيوية. اختُبرت العديد من سلالات هذا الطفيل في جميع أنحاء العالم، واستُخدم بعضها بنجاح في المكافحة الحيوية، في حين كان بعضها الآخر أقلّ كفاءةً. لم يتمّ تعريف أيٍّ من سلالات الفطر *Ampelomyces* سابقاً في سورية، بيد أنّه تمّ تعريف بعض العزلات مورفولوجياً في المنطقة الساحلية، ولم يُشَر إلى وجوده في أيّ منطقة أخرى من سورية. من خلال المسح الحقلي على مدار ثلاث سنوات (2019 – 2021)، تمّ جمع 73 عينة نباتية من 5 محافظات، تضمنت المناطق الساحلية والجنوبية. وُجدت بكنيدات الفطر *A. quisqualis* في 5 عينات من المنطقة الساحلية، وسُجل ظهورٌ غير متوقع للفطر في عينةٍ واحدة من المنطقة الجنوبية. تمّ توثيق وجود هذا الفطر على عائل فطري جديد هو *Golovinomyces neosalviae* المسبب لمرض البياض الدقيقي على نبات المريمية الشائعة *Salvia officinalis*. تمّ الحصول على العزلة S.ham82 لهذا الفطر على الوسط PDA، وقُيّم النشاط التطفلي عن طريق العدوى الاصطناعية باستخدام طريقة الورقة المفصولة ضمن ظروف المختبر. دُرست الصفات الشكلية لهذه العزلة وقُورنت مع العزلة Bah1 من المنطقة الساحلية، إذ تراوح متوسط أبعاد البكنيدات للعزلة S.ham82 ما بين 77.44 (±6.12) X 25.28 (±17.16) ميكرومتر (µm) على العائل الطبيعي و(±40.14) 115.95 X (±42.34) 125.27 ميكرومتر و(±52.41) 167.64 X (±60.06) 189.51 ميكرومتر على الوسط PDA قبل وبعد العدوى الاصطناعية على الترتيب، و(±5.68) 27.98 X (±20.05) 88.24 ميكرون على الأوراق المفصولة الملقحة اصطناعياً. كما بلغ متوسط أبعاد الأبواغ الكونيدية (±0.51) 3.88 X (±0.87) 8.11 ميكرومتر على العائل الطبيعي، و(±0.80) 3.18 X (±1.65) 8.86 ميكرومتر على الوسط PDA قبل وبعد العدوى الاصطناعية، و(±0.37) 3.61 X (±0.69) 7.82 ميكرومتر على الأوراق المفصولة الملقحة اصطناعياً. على حدِّ علمنا، يعدّ هذا البحث التسجيل الأول للظهور الطبيعي للفطر فائق التطفل *A. quisqualis* على العائل الفطري *G. neosalviae* المتطفل على نبات المريمية الشائعة *S. officinalis*.

كلمات مفتاحية: *Ampelomyces* sp.، المريمية الشائعة، *Golovinomyces neosalviae*، سورية.

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