Mycologia Iranica 6(1): 49 – 54, 2019 Short Article DOI: 10.22043/MI.2020.121093

First record of *Epicoccum andropogonis* growing on *Paspalum dilatatum* ergot in Iran

S. Hatami Rad

L. Ebrahimi ✉

Department of Entomology and Plant Pathology, Aburaihan Campus, University of Tehran, Tehran, Iran

H. Shahbazi

Department of Plant Protection, Rice Research Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran

Abstract: *Paspalum dilatatum* spikelets with ergot symptoms were collected from Rice Research Institute of Iran in Rasht, Guilan province during the fall of 2018. Ergot symptoms usually are caused by different *Claviceps* species on grasses. Sclerotia of ergot were globular in shape, black in color and irregularly roughened on the surface that resembles a brain. Different fungi were isolated from the ergot symptoms such as *Alternaria* species mostly. Some isolates were identified as *Epicoccum* based on the morphological features. Morphological characteristics of the isolates were studied on both host substrate and culture media (potato dextrose agar, oat meal agar and malt extract agar) *in vitro*. Shape, color and size of conidia of fungus grown on host substrate (ergot) were similar to the grown conidia on culture media *in vitro* condition. This fungus was identified as *Epicoccum andropogonis* based on molecular data of ITS-rDNA sequence, morphological characteristics and host specificity, which usually grown on *Claviceps* honeydew and immature sclerotia and can be consider as an indicator of ergot disease on grasses. This is the first report of ergot symptoms on *P. dilatatum* as well as *E. andropogonis* species from Iran.

Key words: *Claviceps*, Sclerotia, host, weed

INTRODUCTION

Paspalum dilatatum Poir. or dallisgrass is a perennial grass native to South America that has been introduced into tropical and subtropical areas as a common weed (https://www.cabi.org/isc/datasheet/38953#tosummaryOfInvasiveness).

The genus *Claviceps* Tul. includes 79 species (www.indexfungorum.org) which parasitize only the flowers of specific grasses causing ergot (Alderman et al. 1999). The sclerotia of many *Claviceps* species contain alkaloids (Blaney et al. 2000). *Claviceps paspali* F. Stevens & J.G. Hall causal agent of ergot is only known to colonize *Paspalum* grasses such as *P. dilatatum*, which are important as animal feed (http://toxinology.nilu.no/). This disease has most frequently been observed in the southeastern United States, Central and South America, parts of Europe and South Africa, as well as Australia and New Zealand (Evans & Gupta 2007). This fungus has been recorded on *Brachiaria eruciformis* by Esfandiari in 1948 from Mazandaran province of Iran. Also, *C. microcephala* (Wallr.) Wint. has been reported on *Alopecurus* sp. by Esfandiari (1948) from Kandovan, Iran. *Claviceps purpurea* (Fr.) Tul. has been identified on *Secale cereal* (Ershad 1995), *Agropyron repens* and *Lolium perenne* (Viennot-Bourgin 1958) from Iran.

Another fungus often found in association with ergot was identified as *Cerebella* sp. (Blaney et al. 2000). *Cerebella* Ces. is a black-colored saprophytic mold with a deeply invaginated surface and spherical shape that resembles a brain, and extensively colonizes the honeydew produced by *Claviceps* species. So, the presence of *Cerebella* should be considered only as a sign or indicator of possible ergot that must be confirmed by identification of actual fungal bodies or other structures of *Claviceps* spp. (Alderman et al. 1999). Blaney et al. (2000) study showed that sorghum ergot (*Claviceps africana* Freder., Mantle & De Milliano) contents were mature sclerotia free from floral parts of sorghum, and immature sclerotia with adhering floral parts and with or without attached black sporodochia of *Cerebella*. Also, *Epicoccum andropogonis* (Ces.) Schol-Schwarz (*Cerebella andropogonis* Ces.) conidiomata forming

Submitted 16 Jan. 2019, accepted for publication 25 Feb. 2019
✉ Corresponding Author E-mail: le_ebrahimi@ut.ac.ir

http://mij.areeo.ac.ir

on the honeydew of *C. paspali* in the spikelet of grasses (Ryley et al., (n.d.) in Ergot fungi of Australia (http://collections.daff.qld.gov.au/web/key/ergotfungi /Media/Html/cerebella.html).

In the present study, fungi were isolated from *P. dilatatum* specimens with ergot symptoms and characterized based on morphological and molecular data.

MATERIALS AND METHODS

Samples and fungal isolates

Paspalum dilatatum with ergot symptoms (Fig. 1) were collected from Rice Research Institute of Iran in Rasht, Guilan province in October 27, 2018. Fungal isolation and purification was conducted according to Ebrahimi & Fotouhifar (2016a).

Dried specimens are maintained in the Fungal Reference Collection of the Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN) (accession number: IRAN 17618F). Pure isolates were deposited in the Iranian Fungal Culture Collection (IRAN) at the Iranian Research Institute of Plant Protection, Tehran, Iran (accession number: IRAN 3738C).

Morphological characterization

Culture characteristics were described based on cultures of potato dextrose agar (PDA), oat meal agar (OA) and malt extract agar (MEA) media after 7 and 14 days incubation at 25 °C in dark and under near-UV light source (12 h light/12 h dark). Colony colors (surface and reverse) were assessed using the color charts of Rayner (1970). Microscopic observations were based on the morphological characteristics of conidia and conidiophores on culture media and naturally infected host substrates. Measurements (n = 50) and microphotographs were taken from slides using an Olympus BH2 light microscope (Olympus, Japan).

Molecular characterization

DNA extraction was performed according to the method described in Ebrahimi et al. (2016). Extracted DNA was diluted in 50 µl distilled water and were kept at -20°C for future use. Molecular identification of the fungal isolate was performed based on ITS-rDNA sequence that was amplified using the ITS1/ITS4 primer pair (White et al. 1990). The reaction mixture and PCR condition for ITS was the same as described by Ebrahimi and Fotouhifar (2016b). PCR product of the ITS region was purified and directly sequenced in one direction with ITS1 primer, by Microsynth Company (Microsynth, Switzerland). After sequencing, sequences were manually edited with Chromas 2.4 software (Technelysium, Australia) and the edited sequence was saved in FASTA format.

For phylogenetic analysis, reference sequences of the homologous regions of *Epicoccum* species obtained from GenBank, NCBI (isolates information are provided in Table 1) and then the sequences were aligned with Clustal W (Thompson et al. 1994). *Mycosphaerella rabiei* [accession no. KY788119 (Table 1)] was used as an out-group taxon. Neighbor joining (NJ) analysis (Saitou & Nei 1987) and Maximum likelihood (ML) analysis (Felsenstein 1973) was performed by heuristic search with Mega 7 (Kumar et al. 2016). Bootstrap analysis (Felsenstein 1985) of the ML tree was performed on 1000 replicates. The sequence was deposited in GenBank (NCBI) with accession number MN757870.

RESULTS AND DISCUSSION

Fungal isolates

Different fungal isolates such as *Alternaria* species (mostly) were identified. Five isolates isolate were identified as *Epicoccum* based on morphological features, and one isolate surveyed based on molecular data and identified as *E. andropogonis*, which has been reported as saprophytic fungus on *Claviceps* sp.

Morphology

Culture characteristics- Colonies on OA, PDA and MEA reached 43, 43 and 28 mm in diameter, respectively, after seven days incubation at 25 °C in 12 h dark and 12 h under near-UV light source. Colonies (three replicates) after 14 days on OA were flat, margin regular, with sparse white aerial mycelia, rosy buff to brick color in both side (Fig. 2a). Colonies on PDA margin irregular, aerial mycelia floccose, rust in center (with white dots) to olivaceous grey and cinnamon near to margin; reverse dark brown at center and cinnamon at margin (Fig. 2b). Colonies on MEA margin snaggy, covered by floccose aerial mycelia with some rust dots, vinaceous buff color; reverse black with an umber margin (Fig. 2c).

Fig. 1. *Paspalum dilatatum* specimens. a. with ergot symptoms, b. ergot sclerotium with brain like surface.

Table 1. Strains used in the phylogenetic analysis of *Epicoccum* species.

Species	Isolate	Source	Origin	GeneBank accession no.
Epicoccum andropogonis	CBS 193.55	-	South Africa	MH857441
	CBS 195.55	-	South Africa	MH857443
	IRAN 3738C	***Paspalum dilatatum***	**Iran**	**MN757870**
E. hordei	LC 8148	*Hordeum vulgare*	Australia	KY742097
	LC 8149	*Hordeum vulgare*	Australia	KY742098
E. italicum	CGMCC 3.18361	*Acca sellowiana*	Italy	NR_158264
	LC:8150	*Acca sellowiana*	Italy	KY742099
	P15I5	*Rosa canina*	Iran	MK100172
E. plurivorum	CBS 558.81	*Setaria* sp.	New Zealand	MH861377
	A08	indoor air	Austria	KC248542
	MF-32.32	*Calystegia sepium*	Russia	MH651566
E. pimprinum	CBS 246.60	Soil	India	FJ427049
	PD 77/1028	Soil	India	FJ427050
E. poae	LC 8160	*Poa annua*	USA	KY742113
	LC 8161	*Poa annua*	USA	KY742114
	LC 8162	*Poa annua*	USA	KY742115
E. sorghinum	CBS 179.80	*Sorghum vulgare*	Puerto Rico	FJ427067
	CBS 627.68	*Citrus* sp.	France	FJ427072
	LC 4860	*Camellia sinensis*	China	KY742116
Mycosphaerella rabiei	GRSH102	organic debris	Iran	KY788119

Conidiomata sporodochial, aggregated, superficial and brown. Hyphae septate, branched, 2–4 (3.15) µm. Conidiophores brown with 5–10 (6.8) × 14–30 (18.9) µm in size. Conidia multicellular-phragmosporous, subglobose-pyriform, with a basal cell, dark brown, 8–20 (14.6) × 14–28 (19.4) µm in diameter (Fig. 2d, e).

Morphology on host

Sclerotia of ergot were globular in shape, 2.5–5 (3.9) mm in diameter, black in color and irregularly roughened on the surface that resembles a brain (Fig. 1b) which was according to description of *C. paspali* by Brown (1916).

Unfortunately, none of sclerotia were grown *in vitro* condition to investigate the morphological features and molecular data of the fungus. Based on the ergot symptoms on *P. dilatatum* spikelets and host, and previous similar studies, the causal agent of the ergot symptoms is *Claviceps* cf. *paspali*. This fungus has been recorded on *Brachiaria eruciformis* by Esfandiari (1948) from Iran. This is the first record of ergot on *Paspalum dilatatum* in Iran.

Shape, color and size of conidia of *E. andropogonis* grown on host substrate (ergot) (8–20 (14.75) × 12–24 (19.8) µm) were similar to the cultured conidia on OA *in vitro* condition.

There was not any description of *E. andropogonis* on culture media among literatures to compare with our results in this study. Nevertheless, morphology of conidia *in vitro* was similar to the characteristics of conidia on host substrate, and it was according to the description of this species provided by Ryley et al. (n.d.) in Ergot fungi of Australia (http://collections.daff.qld.gov.au/web/key/ergotfungi/Media/Html/cerebella.html). This is the first record of *E. andropogonis* in Iran.

Specimen examined. IRAN, Guilan province, Rasht, Rice Research Institute of Iran, on *Paspalum dilatatum* spikelet. S. Hatami Rad, L. Ebrahimi & H. Shahbazi, 27 Oct. 2018. Herbarium accession number: IRAN 17618F).

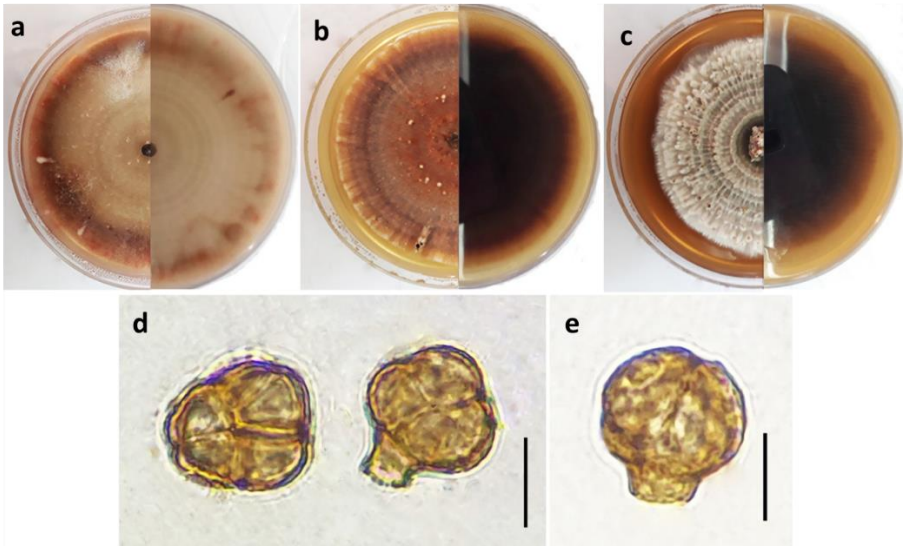

Fig. 2. *Epicoccum andropogonis*. Colony on a. OA, b. PDA, and c. MEA medium, d-e. Conidia formed on OA. — Scale bars = 10 µm.

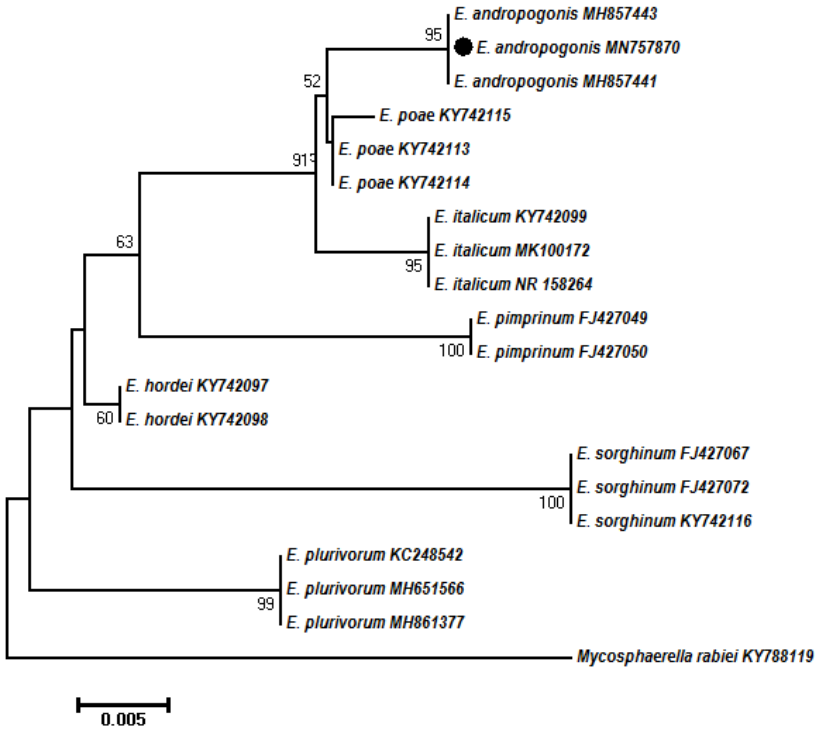

Fig. 3. Neighbor-joining (NJ) tree based on aligned sequences of ITS region of 19 isolates of *Epicoccum* and *Mycosphaerella rabiei* KY788119 as out-group generated in MEGA 7. Bootstrap values (1000 replicates) indicated at the nodes. The scale bar indicates nucleotide substitution in NJ analysis, values ≥50 % are shown above/below the branches.

Molecular analysis

The NCBI BLAST analysis of ITS sequences (with 511 nucleotides) of the *E. andropogonis* isolate (GenBank Accession No. MN757870) obtained from *P. dilatatum* showed a similarity more than 99% with *E. andropogonis* isolates (MH857441 and MH857 443). NJ and ML trees based on aligned sequences of ITS region in 19 isolates (with average of 495 nucleotides) of *Epicoccum* species were generated in MEGA 7. Topologies of the NJ and ML trees were almost similar with respect to identified clades except for minor differences in bootstrap values and only the NJ tree is presented here (Fig. 3). Our isolate is phylogenetically closely related to *E. poae* but differs in the size of epicoccoid conidia (10–23 µm) (Chen et al. 2017).

Phylogenetic analysis confirmed our isolate as *E. andropogonis* as well as morphology and host specificity and clearly revealed its phylogenetic relation with some other species in *Epicoccum*.

ACKNOWLEDGEMENTS

We wish to thank reviewers for their constructive comments. This study was supported by University of Tehran, Iran.

REFERENCES

Alderman S, Frederickson D, Milbrath G, Montes N, Narro-Sanchez J, Odvody G. 1999. A laboratory guide to the identification of Claviceps purpurea and Claviceps Africana in grass and sorghum seed samples. http://www.oda.state.or.us.

Brown HB. 1916. Life history and poisonous properties of Claviceps paspali. Journal of Agricultural Research 7(9): 401–407.

Chen Q, Hou LW, Duan WJ, Crous PW, Cai1 L. 2017. Didymellaceae revisited. Studies in Mycology 87: 105–159.

Ebrahimi L, Fotouhifar Kh-B. 2016a. Identification of some fungi accompanying the scab symptoms in Iran. Mycologia Iranica 3(1): 25–37.

Ebrahimi L, Fotouhifar Kh-B. 2016b. First report of Cyphellophora fusarioides (Chaetothyriales) on a plant host. Sydowia 68: 131–137.

Ebrahimi L, Fotuhifar Kh-B, Javan Nikkhah M, Naghavi M-R, Baisakh N. 2016. Population genetic structure of apple scab (Venturia inaequalis (Cooke) G. Winter) in Iran. PLoS ONE 11(9): e0160737.

Ershad D. 1995. Fungi of Iran. Minirtry of Agriculture, Agricultural Research, Education and Extension Organization, Tehran, Iran.

Esfandiari E. 1948. Troisième liste des fungi ramassés en Iran. Entomologie Phytopath. appl. 8: 1–15.

Evans TJ, Gupta RC. 2007. Veterinary Toxicology Basic and Clinical Principles. Chapter 80: Tremorgenic mycotoxins. Academic press Pp: 1004-1010.

Felsenstein J. 1973. Maximum likelihood and minimum-steps methods for estimating evolutionary

trees from data on discrete characters. Systematic Zoology 22: 240–249.

Felsenstein J. 1985. Confidence intervals on phylogenies: an approach using bootstrap. Evolution 39: 783–791.

Index Fungorum Home Page. www.indexfungorum.org/.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0. for bigger Datasets. Molecular Biology and Evolution 33: 1870–1874.

Paspalum Dilatatum (Dallisgrass), www.cabi.org/isc/datasheet/38953#tosummaryOfInvasiveness.

Rayner RW. 1970. A mycological color chart. Commonwealth Agricultural Bureau, Kew, UK.

Ryley M, Shivas R, McTaggart A, Beasley D. Ergot Fungi of Australia. http://collections.daff.qld.gov.au/web/key/ergotfungi/Media/Html/cerebella.html

Saitou N, Nei M. 1987. The neighbor–joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–80.

Toxinology Norway Home, 2020, http://toxinology.nilu.no/.

Viennot-Bourgin G. 1958. Contribution à la connaissance des champignons parasites de l Iran. Ann. Epiphyt. N. S. 9: 97–210.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innes MA, Gelfand DH, Sninsky JJ, White TJ. (eds) PCR Protocols: A guide to methods and applications, pp. 315–322. Academic Press, New York.

اولین گزارش از گونه *Epicoccum andropogonis* از روی علائم ارگوت در گیاه *Paspalum dilatatum* برای ایران

سپیده حاتمی راد[1]، لیلا ابراهیمی[1]✉، حدیث شهبازی[2]

۱- گروه حشره‌شناسی و بیماری‌های گیاهی، پردیس ابوریحان، دانشگاه تهران، تهران، ایران

۲- بخش گیاه‌پزشکی، موسسه تحقیقات برنج کشور، رشت، ایران

چکیده: سنبله‌های *Paspalum dilatatum* با علائم ارگوت از محوطه موسسه تحقیقات برنج ایران واقع در رشت، استان گیلان در طی پاییز سال ۱۳۹۷ جمع‌آوری شدند. علائم ارگوت معمولا توسط گونه‌های مختلف *Claviceps* روی گندمیان ایجاد می‌شود. اسکلروت‌های ارگوت به شکل گرد، سیاه رنگ و با سطح نامنظم مشابه مغز بودند. قارچ‌های مختلفی از علائم ارگوت جداسازی شدند که بیشتر شامل گونه‌های *Alternaria* بودند. تعدادی از جدایه‌ها بر اساس ویژگی‌های ریخت‌شناختی به عنوان *Epicoccum* شناسایی شدند. ویژگی‌های ریخت‌شاختی جدایه‌ها روی سطح میزبان و همچنین روی محیط‌های کشت (سیب‌زمینی-دکستروز-آگار، عصاره جو دو سر-آگار و عصاره جو-آگار) در شرایط آزمایشگاهی مورد مطالعه قرار گرفت. شکل، رنگ و اندازه کنیدی‌های قارچ روی سطح میزبان (ارگوت) مشابه کنیدی‌های رشد کرده روی محیط‌های کشت در شرایط آزمایشگاهی بود. این قارچ بر اساس اطلاعات مولکولی ناحیه ITS-rDNA، ویژگی‌های ریخت‌شناختی و اختصاصیت میزبانی، که معمولا روی ترشحات قارچی و اسکروت‌های نابالغ رشد می‌کند و می‌تواند به عنوان یک شاخص از بیماری ارگوت روی علف‌ها باشد، به عنوان *E. andropogonis* شناسایی شد. این اولین گزارش از علائم ارگوت روی *P. dilatatum* و گونه *E. andropogonis* از ایران می‌باشد.

کلمات کلیدی: *Claviceps*، اسکلروت، میزبان، گیاه علفی

مکاتبه کننده: لیلا ابراهیمی Email: le_ebrahimi@ut.ac.ir

تاریخ دریافت: ۱۳۹۷/۱۰/۲۶ تاریخ پذیرش: ۱۳۹۷/۱۲/۰۷