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SURVEY of ENTOMOPATHOGENIC FUNGI NATURALLY INFECTING COWPEA APHID, *Aphis craccivora*. KOCH.

Ibrahim, H. Y. E.[1]; A. M. Salam[2]; M. Abdel-Mogib[3] ; M. E. El-nagar[1] ; Hoda A. Salem[2]; Maha S. A. Nada[1]

1. Plant protection Resesarch Institute, ARC, Dokki, Giza, Cairo.
2. Zoology Department, Faculty of Science, Mansoura University.
3. Chemistry Department, Faculty of Science, Mansoura University.

ABSTRACT

Entomopathogenic fungi naturally associated with cowpea aphid, *Aphis. craccivora* were surveyed and identified. There were 18 fungal species infected cowpea aphid, on broad bean in winter and on cowpea in late spring and summer from December (2008) to December (2009). Five genera of entomopathogenic fungi were recorded for the first time associated with *A. craccivora* in Egypt. These genera were: *Trichoderma longibrachiatum, Panadora neoaphidis, Batkoa major, Entomophthora planchoniana, and Coidiobolus obscurus.*

INTRODUCTION

Cowpea aphid, *Aphis craccivora* Koch. is a serious pest having an extensive host range. In addition to cowpea, it infests many other legumes, cotton and as well as Shepherd`-purse, lambsquarters, lettuce, pepperweed, *Polygonum sp.* and *Rumex sp.* It injects a powerful toxin into the plant while feeding and, when populations are large, this can stunt or kill plants. While feeding, this aphid produces a considerable amount of honeydew upon which a sooty mold grows. The black sooty mold reduces photosynthesis and hinders the plant growth. Cowpea aphid transmits nearly 30 plant viruses including cotton curliness virus (Kennedy *et al.*, 1962) and (Blackman and Eastop, 1984). Also, it transmits Peanut stripe virus (PStV) and Peanut mottle virus (PMV). Moreover, it was recorded as a vector of transmission of the Sri Lankan passion fruit mottle virus (Dassanayake and Hicks, 1992); Chili veinal mottle virus and pepper mottle virus (Cerkauskas, 2004).

Entomopathogenic fungi were among the first organisms used as microbial control agents for aphid species. The entomopathogenic fungi have many successes because their characteristics of good epizootic but slow action and over dependence on a suitable environmental factors, which make them useful after establishment. Many of them have relatively wide host ranges among insects. Another advantage is the fact that they do not have to be ingested by the insect host but can invade the host upon contact with the insect cuticle (Boucias *et al.*, 1988).

The present study was carried out to record and identify the entomopathogenic fungi naturally associated with cowpea aphid to be focused in order to be used as microbial control agents in the future.

MATERIALS AND METHODS

Survey of entomopathogenic fungi associated with *A. craccivora*:

The survey was conducted in Dakahlia Governorate during 2009. The survey was monthly carried out on broad bean in winter and cowpea at late spring and summer.

Leaves attached to cadavers of dead insects and/or those showing any symptoms of fungal infection were collected in plastic bags and transferred to the laboratory. The collected dead insects were kept in Petri-dishes prepared with moisted filter paper at 25± 2 C^0 and examined daily if any mycoses symptoms were indicated. The dead insects were mounted with lactophenol and examined microscopically.

Isolation of the entomopathogenic fungi:

Aphids cadavers showing natural growth of fungi were collected and then cultivated on autoclaved Sabouraud dextrose yeast extract agar (SADYA) [10g/l peptone, 40g/l dextrose, 10g/l yeast extract and 20g/l agar] and incubated at 25± 2 C^0 and 80 ±5% RH until further growth. After obtaining cultures of fungi, cultures were then purified using single spore or hyphal tip technique.

Also,autoclaved Sabouraud dextrose yeast agar supplemented with egg yolk and milk [(SADYA), 80 ml/l egg yolk(4-5 eggs) and 120ml/l sterilizing milk] was used as specific media for cultivating Entomophthorales, because they need rich nutrients for growing. These nutrients have been supplied by this media. The whole ingredients except egg yolk and milk were added to the boiling water, then were be autoclaved at 120 C^0 for 20 min. then maintained at 50-60 C^0. Fresh eggs were sterilized in a mixture of 90% alcohol (200ml) and 2% sodium hypochlorite (800ml).The egg shells were breaded near the flame of a Bunsen burner. Eggs yolk was gently separated and poured into a sterile graduated cylinder. Sterilizing milk (at room temp.) was added to egg yolk, then the contents were stirred with a sterile glass rod till became homogeneous. The egg yolk milk mixture was added to the autoclaved dextrose yeast agar medium with adding five g/l Ampicillin, then shacked. Immediately, the medium was poured into sterile Petri dishes and kept under 4 C^0. (Papierok and Hajek, 1997).

Identification of the entomopathogenic fungi:

Aphids cadavers or portion of them were mounted on slides stained with lactophenol cotton blue and examined under light microscope as well as cultivated on the artificial media, then examined to insure maintenance of the same fungus.

The isolated fungi were identified based on the keys of Humber (1997) by mycologists from Assiut University Mycological Center (AUMC),Egypt; and Dr. Maha Salah El-Din Ali Nada, Plant protection Resesarch Institute, ARC, Dokki, Giza, Cairo.

RESULTS AND DISCUSSION

The present study recorded 18 fungal species infected cowpea aphid, *A. craccivora* on broad bean in winter and on cowpea in late spring and summer from December (2008) to December (2009) as shown in Table(1).

Table (1): Fungal species infected cowpea aphid, *A. craccivora* on broad bean in winter and on cowpea in late spring and summer from December (2008) to December (2009) in Dakahlia governorate.

Host plants	Fungi	Date
Broad bean	*Cladosporium cladosporoides*, (Fig.1)	January, February, March and April
	Trichoderma longibrachiatum, Fig. (2)	February, March and April.
	Epicoccum spp., Fig. (8).	March and April.
	Penicillium oxalicum (Currie & Thom), Fig. (9).	March and April.
	Panadora neoaphidis, Fig. (3).	December (2008), January, February, March, December (2009)
	Batkoa major, Fig. (4).	March and April.
	Neozygites fresenii, Fig. (6).	December(2008) December (2009)
	Entomophthora planchoniana, Fig. (5).	January, February, March
	Conidiobolus obscurus, Fig. (7).	April
	Fusarium semitectum Berkely, Fig. (11).	April
	Aspergillus flavus, Fig. (13).	April
	Aspergillus niger, Fig. (12).	April
	Verticillium spp., Fig. (10).	January, February
	Alternaria spp, Fig. (17).	March and April.
	Mucor spp. Fig.(18).	March and April.
Cowpea	*Cladosporium cladosporoides*, Fig. (1).	May and June.
	Trichoderma longibrachiatum, Fig. (2).	May and June.
	Coidiobolus obscurus, Fig. (7).	May and June.
	Epicoccum spp., Fig. (8).	May.
	Penicillium oxalicum (Currie & Thom.), Fig. (9).	April, May and June.
	Fusarium semitectum Berk.&Rav., Fig. (11).	May and June.
	Aspergillus flavus, Fig. (13).	May, June and July.
	Aspergillus niger, Fig. (12).	April, May, June and July.
	Aspergillus terrus, Fig. (14).	July and August.
	Eurotium omstelodami Mangin., Fig. (15).	May and June.
	Nigrospora oryzae (Berkeley & Broome)., Fig. (16).	June.

Five genera of promising entomopathogenic fungi were recorded for the first time associated with *A.craccivora* in Egypt. These genera were:

- *T. longibrachiatum*: Septate hyaline hyphae, conidiophores, phialides, and conidia are present; it produce chlamydospores; Phialides are hyaline, branched, flask – shaped, inflated at the base, solitary or may appear in clusters, and are attached to the conidiophores at right angles; Conidiophores are hyaline, branched, and may occasionally demonstrate a pyramidal arrangement; and conidia are unicellular, round or ellipsoidal, green in color, smooth walled or rough, with an average diameter of 3 µm, and are grouped in sticky heads at the tips of the

phialides, however, these clusters usually get disrupted during slide preparation procedure intended for microscopic examination. Cadavers were black in color becoming grey when dried Fig. (2).

- *P. neoaphidis*:

Conidiophores are digitately branched at their apices. Primary conidia are clavate to obovoid, uninucleate with basal papillae discharged laterally from the spore axis, forcibly discharged by papillary eversion. Secondary conidia nearly globose and they are produced on primary conidia. Cystidia tapering toward bluntly pointed apex, thicker than hyphae at base. Rhizoids have prominent terminal discoid holdfast. Freshly killed cadavers showing typically pale brown in color then turning a rusty red color upon desiccation.Fig. (3).

- *B. major*: Conidiophores are simple with narrow neck between conidium and conidiogenous cell. Primary and secondary conidia are globose, multinucleate, discharged by papilla reversion. Papilla has pointed extension. Rhizoids are with terminal discoid holdfasts. Resting spores are bud laterally from parental hyphae; with unfixed nuclei. Cadavers becoming white in color, the conidia follow the body especially intrasegmental areas of the abdomen and wings. Fig. (4).
- *Ento. planchoniana*: Conidiophores are simple unbranched. Conidiogenous cells are club-shaped. Primary conidia are bell-shaped, with broad flat papilla and pointed apex, forcibly discharged. Secondary conidia are budding from the primary conidia, slightly smaller, nonapiculate with more rounded papillae. Rhizoids are numerous, isolated and have the same diameter of conidiophores.Freshly killed aphids typically brick red in color becoming a pale brown color upon sporulation. Fig. (5).
- *Coni. obscurus:* Conidia globose, hemispherical papilla emerges abruptly from spore outline; no capilliconidia or microconidia formed. The mature resting spores characterized by their thick cell walls.Cadavers were surrounded with few white mycelium growths. Fig. (7).

Also, the fungus, *Neo. fresenii* which was recorded for the third time in Egypt and for the first time associated with *A.craccivora* in Dakahlia governorate. It was recorded by Sewify (2000) at the first time in Giza Governorate, followed by Nada (2006) in Sharkia Governorate. Conidiophores are simple unbranched. Primary conidium is nearly spherical to ovoid with a flattened basal papilla, varying in size and forcibly dicharged. Secondary conidia are capilliconidia carried on capillary conidiophores arising from primary conidia, varying in size , passively discharged from capillary conidiophores and almond-shaped with a mucoid drop at the tip. Resting spores are black to smoky- gray in color arising from conjugation between two spherical gametangia. Dried cadavers were light tan or gold brown in color, smooth and flattened. The mycosed aphids were typically dark brown to gray with a shade of violet in color. Fig. (6).

Moreover, *C. cladosporides*, which was recorded by Abdel-Baky (2000) in Dakahlia Governorate followed by Abd-Allah (2004) in the same governorate. Colonies are mostly olivaceous-green to olivaceous- brown,

velvety; reverse olivaceous- black. Conidiophores are branching acropleurogenously and bearing numerous conidial chains arising below septa, but without swellings and sympodial elongations. Conidia ellipsoidal to lemon shaped, mostly smooth-walled, rarely minutely verruculose, olivaceous-brown. Freshly killed cadavers were black in color then becoming olivaceous-grey. Fig. (1).

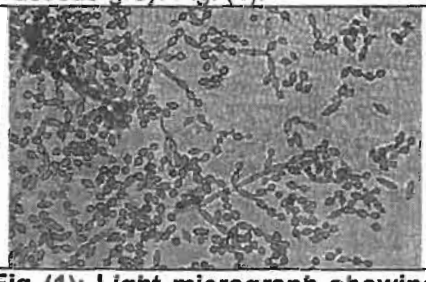

Fig (1): Light micrograph showing branching conidiophores of *C. cladosporoides* bearing numerous conidial chains. Conidia ellipsoidal to lemon shaped.

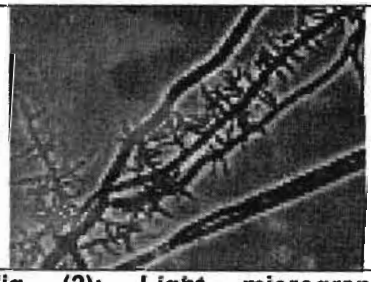

Fig (2): Light micrograph showing *T. longibrachiatum*. Hyaline, branched conidiophores. conidia are unicellular, round or ellipsoidal (arrow).

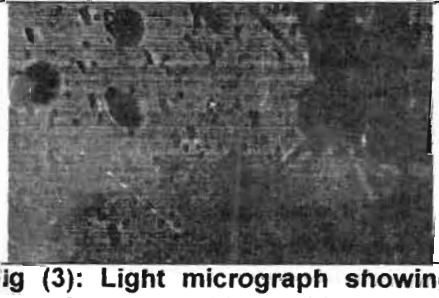

Fig (3): Light micrograph showing primary ovoid conidia of *P. neoaphides*.

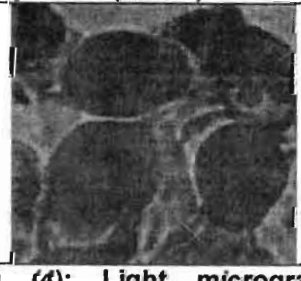

Fig (4): Light micrograph showing *B. major*. Secondary conidia discharged from the primary one.

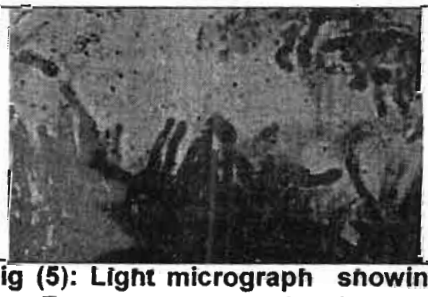

Fig (5): Light micrograph showing *Ento. planchoniana*, unbranched conidiophores and primary conidia.

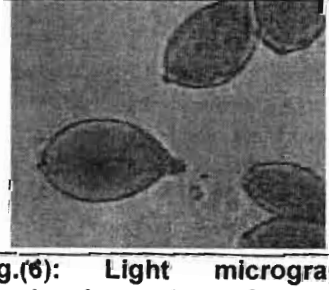

Fig.(6): Light micrograph showing *Neo. fressenii*, almond- shaped capilli-conidium with a mucoid apicaldroplet .

Fig (7): Light micrograph showing mature resting spores of *Coni. obscurus* with thick cell wall

Fig (8): Light micrograph showing *Epicoccum sp.* The globose or pyriform conidia with a funnel-shaped base and broad attachment scar.

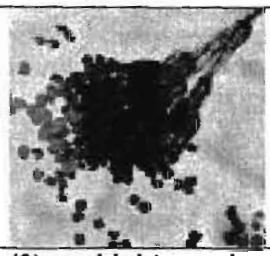

Fig (9): Light micrograph showing *P. oxalicum* with cylindrical phialides, tip distinctly tapring, clusters of conidia which appear silky, strongly ellipsoidal, smooth walled.

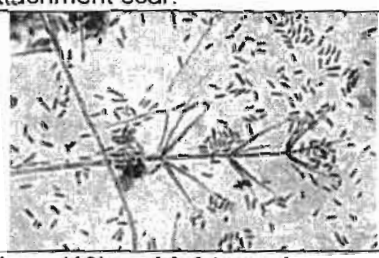

Fig (10): Light micrograph showing *Verticillium sp.* with mucoid conidial balls formed apically on individual phialides.

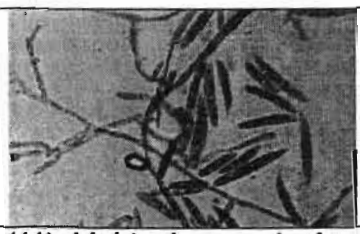

Fig (11): Light micrograph showing *F. semitectum* , with fusiform conidia, almost straight with slightly bent, beaked apical and conical, hardly apiculate, basal cells. Micro-conidia absent.

Fig (12): Light micrograph showing *A. niger.* conidiophores arising from long, broad, thick-walled foot cells. Conidia in large, radiating heads, mostly globose, irregularly roughened

Fig (13): Light micrograph showing *A. flavus*, with radiating conidial heads. on larger conidiophores a layer of metulae supports the phialides. Conidia are globose to subglobose and conspicuously echinulate.

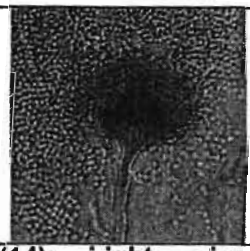

Fig (14): Light micrograph showing *A. terrus*. conidiophores are smooth-walleda and hyaline with hemispherical vesicles. Conidia are globose to ellipsoidal and smooth-walled.

Fig (15): Light micrograph showing *E. amstelodami* .Ascospores are with broad ridges. conidia subglobose to ellipsoidal, with ends commonly flattened and finely papillate

Fig (16): Light micrograph of *N. oryzae* showing flask-shaped conidiophores which arise as short lateral branches from prostrate vegetative hyphae. Conidia are solitary, prolate, black, opaque, smooth-walled.

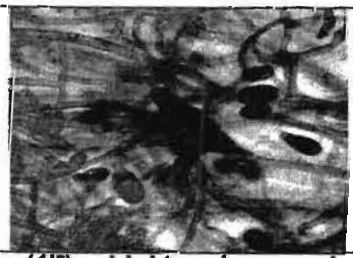

Fig (17): Light micrograph of *Alternaria sp.* Showing conidia catenate, or solitary, dry, typically ovoid or obclavate, smooth or verrucose, with transverse and frequently also oblique.

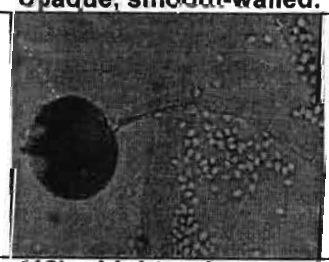

Fig (18): Light micrograph of *Mucor sp.* Showing sporangiophores, forming large, terminal, globose to spherical, multispored sporangia, without apophyses and with well-developed subtending columellae. porangiospores are hyaline, grey or brownish, globose to ellipsoidal, and smooth-walled or finely ornamented.

REFERENCES

Abd-Allah, R. R. H. (2004). Studies on some bioagents against certain sucking insect pests. Ph.D., Thesis, Fac. Agric., Zagazig Univ., 73 pp.

Abdel-Baky, N.F.(2000). *Cladosporium spp.* An entomopathogenic fungus for controlling whitefly and aphid in Egypt. Pakistan Journal of Biol. Sci. 3(10): 1662-1667.

Blackman, R.L.; and Eastop, V. F. (1984) "Aphids on the world`s crops: An identification and information Guide". Wiley, New York.

Boucias, D. G.; Pendland, J. C. and Latge, J. P. (1988). Nonspecific factors involved in the attachment of entomopathogenic Deuteromycetes to host insect cuticle. Appl. Environ. Microbial. 54(7), 1795- 1805.

Cerkauskas (2004). AVRDC- The World Vegetable Center, Fact Sheet, http://WWW.Avrdc.org.

Dassanayake, E. M. and Hicks, R.G.T. (1992). Sri Lanka Passion fruit mottle virus, a potyvirus infecting golden passion fruit in Sri Lanka. Ann. Appl. Bio., 120: 459-169.

Humber, R. A. (1997). Fungi: identification. In: Lacey. L. (ed.), "Manual of Techniquesin insect pathology". Academic press, San Diego, CA, pp. 153-186.

Kennedy, J.S.; Day, M.F. and Eastop, V. F. (1962). A conspectus of aphids as vectors of plant viruses. Commonwealth Institute of Ent., London, 114 pp.

Nada, M.S.E.(2006). Sucking insects infesting some crops and their controlling with entomopathogenic fungi in North Africa. Ph.D., Thesis, Fac. Agric., Cairo Univ., 44 pp.

Papierok, B. and Hajek, A. (1997). Fungi: Entomophthorales. In: Lacey, L. A. (ed.) "Manual of Techniques in Insect Pathology". Academic Press, London, pp. 187-212.

Sewify, G.H. (2000). Neozygites fresenii causing an epizootic in aphid Aphis craccivora Kock. On Faba bean in Egypt. Bull. Fac. Agric., Cairo Univ., 51: 85-94.

حصر الفطريات الممرضة للحشرات التى تصيب من اللوبيا طبيعيًا

هبه يوسف السيد إبراهيم[1]، عبد الرءوف محمد سلام[2]، ممدوح عبد المجيب[3]، محمود السيد النجار[1]، هدى عبد العزيز سالم[2] و مها صلاح الدين على ندا[1]

١. معهد بحوث وقاية النباتات – مركز البحوث الزراعية– الدقى– الجيزة– مصر.

٢. قسم علم الحيوان – كلية العلوم – جامعة المنصورة.

٣. قسم الكيمياء – كلية العلوم – جامعة المنصورة.

تم حصر الفطريات الممرضة للحشرات والتى تتواجد طبيعيًا مع من اللوبيا و تصيبه بداية من شهر ديسمبر ٢٠٠٨ وحتى ديسمبر ٢٠٠٩. وجد أن هناك ١٨ نوعًا من الفطريات يصيب من اللوبيا طول العام؛ فى الشتاء على نبات الفول وفى الصيف ونهاية الربيع على نبات اللوبيا. وقد تم عزل وتعريف هذه الفطريات وتم تسجيل خمسة أنواع لأول مرة فى جمهورية مصر العربية وهى:

Trichoderma longibrachiatum, Panadora neoaphidis, Batkoa major, Entomophthora planchoniana, and Conidiobolus obscurus.

قام بتحكيم البحث

أ.د / عبد البديع عبد الحميد غاتم — كلية الزراعة – جامعة المنصورة

أ.د / هدى عبد الحسيب احمد — كلية العلوم – جامعة المنصورة