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Research Article

Natural occurrence of entomopathogenic fungus, *Cladosporium cladosporioides* on blow fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) on ber in Punjab, India

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ABSTRACT: Surveys and surveillances were carried out in the ber growing regions of Punjab through fixed plot surveys in Fruit Research Farm, Punjab Agricultural University (PAU), Ludhiana, Punjab during 2018 and 2019 to record the emerging insect pests and their natural enemies. During these surveys, infection of a fungus was observed on the adults of blow fly, *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) occurring on ber plants in PAU, Ludhiana. This fly is economically significant as pollinator of fruit crops. The adult flies were observed to be dead and were covered with fungal mycelial growth. Later on, the fungus was identified as an entomopathogenic fungus, *Cladosporium cladosporioides* based on morphological and molecular characterization. This is the first record of infection of an entomopathogenic fungus, *C. cladosporioides* on this pollinator dipteran fly, *C. megacephala* from Punjab, India. Thus, the potential of *C. cladosporioides* for the control of other dipteran insect-pests of fruit crops can be explored.

KEY WORDS: Ber, Biocontrol, Blow Fly, *Cladosporium cladosporioides*, Entomopathogenic Fungus, Pollinator

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INTRODUCTION

Blow fly, *Chrysomya megacephala* (Fabricius) is widely distributed and occurs in every continent from Oriental and Australasian regions, Indian region and the Middle East and has spread dramatically through Africa (Akbarzadeh *et al*., 2015). This is a species of Calliphoridae family and is economically significant as pollinator of mango fruit crop in Taiwan, Australia and Israel (Anderson *et al*., 1982; Hu *et al*., 1995; Dag and Gazit, 2001; Sung *et al*., 2006). On ber, this dipteran fly is known to forage both for pollen and nectar and thus it plays an important role in the pollination of ber fruit crop (Devi *et al*., 1989). The eggs and larvae of *C. megacephala* are parasitized by a number of parasitoids such as *Brachymeria podagrica* (Fabricius) (Chalcididae), *Tachinaephagus zealandicus* Ashmead (Encyrtidae), *Nasonia vitripennis* (Walker) (Pteromalidae) and *Pachycrepoideus vindemiae* (Rondani) (Pteromalidae) (Marchiori, 2004; Devi *et al*., 2010). In Punjab, this fly has been observed as a pollinator on many fruit crops including ber.

Entomopathogenic microorganisms form the basis of bio-insecticides or biological insecticides. They are highly specific, environment friendly, have lower induction of insect resistance and self-sustained in the environment. The main disadvantages include greater susceptibility to environmental conditions and shorter shelf life, which may be minimized through good formulations (Angelo *et al*., 2010). The Entomo-Pathogenic Fungus (EPF), *Cladosporium cladosporioides* has been reported to infect different species of insects such as aphid, *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) (Abdelaziz *et al*., 2018); European pepper moth, *Duponchelia fovealis* (Zeller) (Lepidoptera: Crambidae) (Amatuzzi *et al*., 2018); sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Abdel-baky, 2000); two-spotted spider mite, *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) (Habashy *et al*., 2016; Eken and Hayat, 2009); carmine spider mite, *Tetranychus cinnabarinus* Boisduval (Trombidiformes: Tetranychidae) (Eken and Hayat, 2009); and cotton bollworm, *Helicoverpa armigera* (Hubner) (Bahar *et al*., 2011).

The present study was planned with an objective to record the emerging insect pests on ber trees and their biological control agents in Punjab.

MATERIALS AND METHODS

During 2018 and 2019, surveys were conducted in the ber growing regions of Punjab along with fixed plot surveys in the Fruit Research Farm, Punjab Agricultural University, Ludhiana to record the incidence of insect pests

and their natural enemies. During November 2018 and 2019, a fungal growth was observed on the cuticle of adult blow fly, *Chrysomya megacephala* on the ber trees. A number of flies were found dead and infected with this brownish fungal mycelium. The dead adult blow flies with such fungal growth were collected and sent to ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India for identification.

Infected adults were surface sterilized using 4% Sodium hypochlorite and later rinsed three times with sterile water. The insect bits were plated on Sabouraud's Dextrose Yeast extract broth (SDYA) medium (Dextrose 20 g, mycological peptone 10 g, yeast extract 5 g, in 1L distilled water) and the plates were incubated at 26 ± 1^{0}C for one week for fungal growth. The pure culture was used for the colony morphology and microscopic characteristics.

The genomic DNA was extracted from the 10 days old fungal culture grown on SDYA media using CTAB DNA extraction kit (Himedia) and subjected to PCR.

The ITS region universal primers of ITS1 (5' TCC GTA GGT GAA CCT TGC GG 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') were used for PCR study. The 50 µl of master mix consisting of 50 ng DNA, 10 pmol of Forward and Reverse primer, 1.25 mM of each dATP, dGTP, dTTP, dCTP, 3 units of *Taq* DNA polymerase, 10x Tag buffer with 2.5 mM $MgCl_2$ and sterile de-ionized water. The DNA amplification was carried out in a thermocycler (BioRad) with steps as initial denaturation at 95ºC for 5 min and denaturation at 94ºC for 1 min, annealing at 52ºC for 1 min, extension at 72ºC for 1 min for 35 cycles and final extension at 72ºC for 10 min and stored at 4ºC. The amplified PCR product was sequenced and blasted for identification in National Center for Biotechnology Information (NCBI), USA.

RESULTS AND DISCUSSION

During 2018 and 2019, brown or dark green coloured mycelium was visible on the body of flies and body was flattened from the abdominal region (Fig. 1), enlarged and wrinkled. The cadavers were completely covered with mycelial growth of fungus and their colour changed to whitish green (Fig. 2).

The entomopathogenic fungus observed on the adults of *Chrysomya megacephala* was identified as *Cladosporium cladosporioides* based on the morphology and molecular characterization.

The colony on SDYA medium is olive- dark green to dark brown, slightly greyish and reverse blackish. The

Fig. 1. Infected blow fly cadaver

Fig. 2. Fungal mycelial growth on fly cadaver

colonies diffuse and the mycelia form mats and rarely grow upwards from the surface of the colony. In microscope, darkly-pigmented hyphae are sparse, unbranched or sparingly branched, wide, septate. Conidiophores are narrowly cylindrical to cylindrical-oblong, non-nodulose, unbranched or occasionally branched. Conidia are numerous, branches in all directions, small terminal conidia, sub-globose, ovoid-fusiform (Fig. 3).

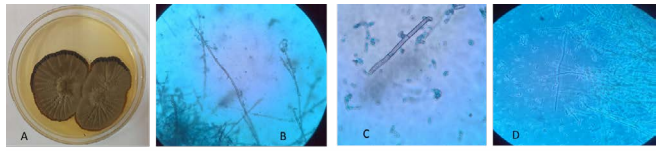

Fig. 3. *Cladosporium cladosporioides* A. Colony growth on SDYA medium; B-D. Hyphae, conidiophores and conidia

The 18S rDNA and ITS nucleotide sequence showed 550bp in length. The sequence was subjected in BLAST program and checked in Genbank database for similarities

which showed 99% homology with similar sequences of *C. cladosporioides* (MH425309 and LC317545). The nucleotide sequence was deposited in NCBI GenBank and the accession number MT645706 was obtained.

Farias and Filho (1987) found that *Cladosporium* spp. was the most important fungus isolated from nymphs of *Aleurothrixusa epium* in cassava plants. Pan *et al*. (1989) reported *C. cladosporioides* as pathogenic to scale, *Hemiberlesiapity sophila* Tagaki causing 39% mortality in laboratory and 20-57% in field conditions. About 18-19% infection of eggs, 47% of nymphs and 50-53% infection of adults of *Bemesia tabaci* with *C. cladosporioides* have been reported in Egypt (Abdel-baky *et al*., 1998). Natural epizootics of *Cladosporium* spp. were observed at the end of summer which may be attributed to the optimum temperatures, high relative humidity and rainfall of that year. Natural occurrence of *C. cladosporioides* on *Tetranychus urticae* mite have been recorded from Tamil Nadu, India (Jeyarani *et al*., 2011). Another *Cladosporium* spp. is known to produce toxins having lethal effect on *Spodoptera litura* (Fabricius) (Singh *et al*., 2016). Two compounds isolated from *C. cladosporioides* caused 100% mortality of aphid, *Aphis gossypii* Glover (Shaker *et al*., 2019).

Cladosporium oxysporum is known to cause epizootics in the populations of mealybug, *Planococcus citri* (Risso) and aphid, *A. gossypii* on guava trees in Eastern Transvaal (Samways, 1983). Abdel-baky and Abdel-salam (2003) observed the seasonal distribution of *Cladosporium* during all year round but higher infection of whiteflies and aphids was observed during June until November. Another fungus, *Beauveria bassiana* has also been reported to be pathogenic to *C. megacephala* (Mehdi, 2018). Some species in the genus *Cladosporium* have been reported to be pathogenic against eggs and larvae of *H. armigera* (Hubner), aphids and silverleaf whitefly (Bahar *et al*., 2011). Similarly, Islam *et al.* (2019) reported *C. cladosporioides* as a potential candidate for control of whitefly, *B. tabaci*.

This is the first report of infection of blow fly, *C. megacephala* by EPF, *C. cladosporioides* in ber orchards in Punjab, India. Since blow flies are pollinators of fruit crops, mainly of ber and mango, the susceptibility of this insect to an EPF is not desirable. This EPF has the potential to act as biological control agent and thus can be tested against other dipteran insect pests of fruit crops.

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