Short Communication

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MANAGEMENT OF ANTHRACNOSE OF BANANA BY UV IRRADIATION

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ABSTRACT

Anthracnose is a postharvest disease of banana *Musa acuminata* Colla caused by the fungus *Colletotrichum musae* Berk & Curtis. The present study was carried out to manage this pathogen *in vitro* and *in vivo* using different types of ultraviolet **(**UV) radiations. Two *in vitro* bioassays were carried out. In the first bioassay, malt extract agar medium was used in 9-cm diameter Petri plates. Plates were exposed for 15, 30, 45,..,120 minutes to three types of UV radiations viz. UV-A of wavelength 365 nm, UV-B of wavelength 302 nm and UV-C of wavelength 254 nm. In the second bioassay, malt extract broth was used in 250-mL conical flasks with the same treatments of UV irradiation. UV-C radiation exhibited the highest fungicidal potential in the two *in vitro* bioassays. UV-C irradiation for 45 minutes and above completely inhibited the fungal growth. Different doses of UV-B radiation reduced fungal biomass and radial growth by 15–75% and 34–75% in malt extract broth and malt extract agar media over control (light), respectively. The effect of UV-A radiation was generally insignificant. *In vivo* bioassays were carried out by exposing *C. musae* inoculated banana fruits to the three types of UV radiations for two hours. UV-C irradiation completely inhibited the fungal growth.

Key words: Anthracnose; Banana; *Colletotrichum musae*; UV irradiation.

INTRODUCTION

Banana, originated from Southeast Asia, is an important food crop that is widely cultivated in tropical and subtropical areas of the world (Zhang *et al.,* 2005). The worldwide demand for bananas is increasing and banana for export must be free from exotic diseases, fungal toxins and chemical residues. Anthracnose, an important post-harvest rot disease of banana, caused by the fungus *Colletotrichum musae*, affects the fruit quality and marketability (Su *et al.,* 2011; Ara *et al.,* 2012). *C. musae* is also the causal agent of crown rot, stem-end rot and blossom-end rot in bananas (Sangeetha *et al.,* 2010). Symptoms of anthracnose appear on green banana fruit as brown to black diamond-shaped lesions. Orange or salmon colored rings may occur on severely infected bananas. On yellowing fruit, brown spots initially appear that later become sunken and covered with orange spore masses. A tip rot may develop and can rot the entire fruit but the pulp is usually not affected unless the fruit is overripe (Stover, 1972 and (Chillet *et al.*, 2007).).

Postharvest diseases are generally controlled by pre- as well as postharvest applications of fungicides (Gachango *et al.,* 2012). However, nowadays producers have sto seek alternative strategies because of increased global demand for chemical-free fresh produce (Korsten, 2006), development of fungicide-resistant strains of phytopathogens (Okada and Furukawa, 2008; Saito *et al.,* 2008) and increased costs of synthetic fungicides (De Costa and Gunawardhana, 2012). UV irradiation is one of presently available non-fungicidal strategies to control postharvest diseases (Canale *et al.,* 2011; Darras *et al.,* 2012). UV radiations are electromagnetic in nature and range between 10 – 400 nm with 3 to 124 eV energies. These radiations have both useful and damaging effects. UV rays can be subdivided into three different wavelength bands UV-A or long wave (400-315 nm), UV-B or medium wave (315-280 nm), and UV-C or short wave (< 280 nm). The present study was carried out to investigate the effect of different types of UV radiations on *in vitro* and *in vivo* growth of *Colletotrichum musae.*

MATERIALS AND METHODS

Isolation of *C. musae*: Banana were purchased from local market from Al-Riyadh, Saudi Arabia and incubated at 15-20 ºC for 7 days to encourage the growth of *C. musae*. Fungus that grew on the fruit surface was harvested using sterile forceps and inoculated onto malt extract agar plates. The isolated fungus was identified as *C. musae.*

In vitro* control of *C. musae: Two experiments were carried out to investigate the effect of UV irradiation on *in vitro* growth of *C. musae*. First experiment was carried out in 9-cm diameter using malt extract agar (MEA) medium. Disks of 5 mm diameter waere cut from the stock culture and inoculated in MEA plates. The plates were exposed to different UV radiations viz. UV-A of wavelength 365 nm, UV-B of wavelength 302 nm and UV-C of wavelength 254 nm for 15, 30, 45, 120 minutes. Each treatment was replicated thrice. For control treatment, the Petri plates were kept in dark as well as in light. After the exposure, plates were transferred to the incubator to observe the growth rate of the test fungus.

The data were recorded after seven days and colony diameter was measured.

Second experiment was carried out using malt extract broth as growth medium. Fifty milliliters of malt extract broth was poured in 250 mL flasks and inoculated with 5 mm diameter disk cut from the tips of actively growing fungal culture. Inoculated flasks were exposed to same UV treatments as in the first experiment. After 7 days of incubation, fungal biomass from each flask was collected pre-weighed filter papers and oven dried to constant weight.

In vivo* control of *C. musae: For *in vivo* studies banana fruits were surface disinfected with 5% sodium hypochlorite solution for 3 min, washed thrice with sterilized distilled water and air dried. Surface sterilized fruits were wounded with a sterilized cork borer (5 mm diameter and 5 mm depth from the surface) and using surgical blade. Three wounds were made per fruit and each wound was inoculated with 50 µL of conidial suspension of the fungal pathogen. The fruits were exposed to UV-A 365 nm, UV-B 302 nm and UV-C 254 nm for2 hours. The inoculated fruits were placed on plastic trays and enclosed in clean plastic bags to maintain high humidity and incubated at room temperature (27 ± 1°C) for one week. After 7 days of storage, disease severity was recorded by measuring their lesion diameters. Conidial suspensions of *C. musae* without any UV treatment served as control.

Statistical analysis: All the data were analyzed by analysis of variance followed by Student Newman Keuls Test at $P \leq 0.05$ using computer software COSTAT.

RESULTS AND DISCUSSION

Effect of UV irradiation on *in vitro* growth of *C. musae:* UV-A irradiation was found to be the least effective in both the *in vitro* bioassays. None of the UV-A treatment exhibited significant effect on fungal biomass in malt extract broth bioassay. However, the highest dose of 120 minutes exposure to UV-A radiation in malt extract agar bioassay significantly reduced fungal radial growth (Fig. 1A & 2A).

UV-B radiation proved moderately effective in controlling fungal growth. There was a gradual decrease in fungal biomass and colony diameter with increase in exposure time in the two bioassays. The effect of all the exposure times was significant over control (light) in both the bioassays. There was 15–75% and 34–75% reduction in fungal biomass and fungal radial growth in malt extract broth and malt extract agar media over control (light), respectively. (Fig. 1B & 2B). Sensitivity of other fungal species namely *Beauveria bassiana*, *Engyodontium albus*, *Simplicillium lanosoniveum* and *Lecanicillium aphanocladii* has also been reported earlier (Braga *et al.*, 2002; Fernandes *et al.*, 2007).

UV-C irradiation exhibited the highest fungicidal effect. In both the bioassays, exposure of the fungus to this radiation for 45 minutes and above completely inhibited the fungal growth (Fig. 1C & 2C). In a recent study, Hamanaka *et al.* (2011) reported that over 90% of *Cladosporium* and 75% of *Penicillium* spores were inactivated by 30 sec of UV-C irradiation. Both the fungi were isolated from the peach fruit. Irradiation of *Botrytis cinerea* cultures with UV-C (254 nm) resulted in 10-fold decline of conidial germination as compared to control (Darras *et al.*, 2012). Siddiqui *et al.* (2011) reported that exposure of mungbean and ground nut seeds for 60 minutes significantly reduced infection by root infecting fungi namely *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fussarium* spp. UV irradiation affects DNA by initiating thymine dimerisation (Shintani, 2003).

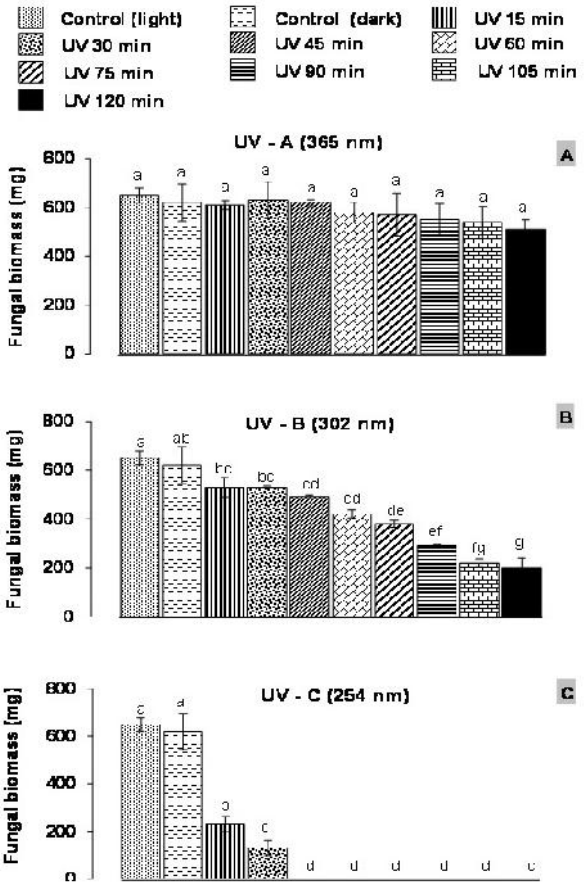

Fig. 1. Effect of different UV radiations on biomass of *Collelotrichum musae* grown in malt extract broth. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Student Newman Keuls Test.

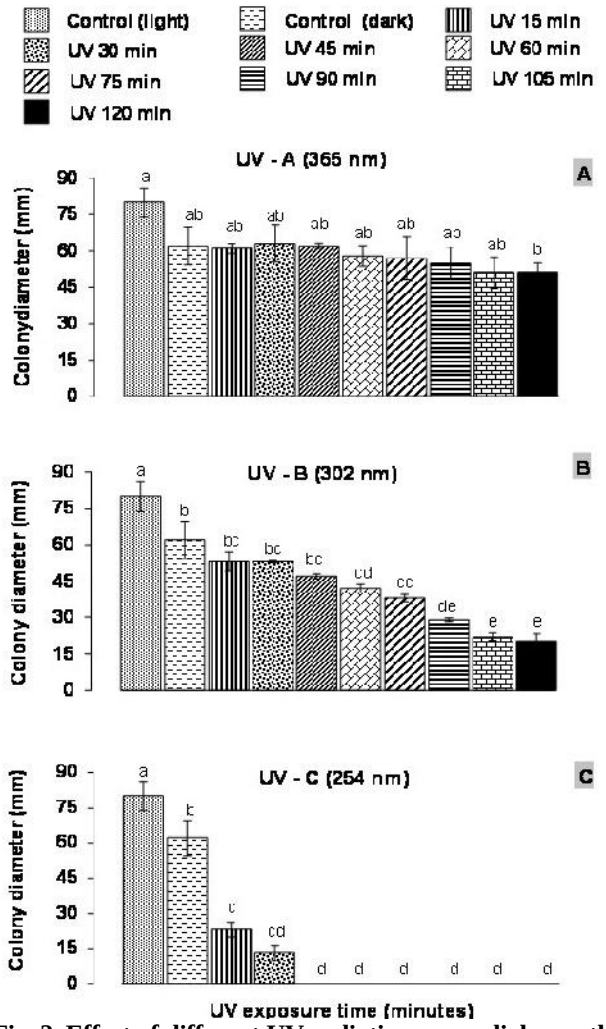

Fig. 2. Effect of different UV radiations on radial growth of *Collelotrichum musae* grown on malt extract agar medium. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Student Newman Keuls Test.

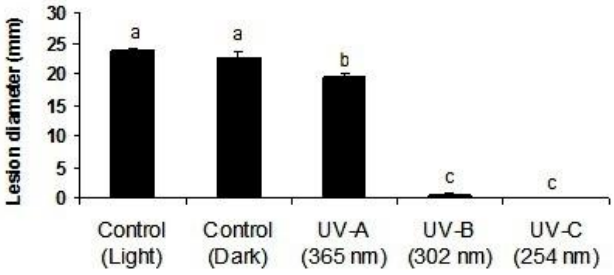

Fig. 3: Effect of UV exposure for 2 hours on lesion diameter formed by anthracnose fungus *Collelotrichum musae* on banana. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Student Newman Keuls Test.

Effect of UV irradiation on *in vivo* growth of *C. musae:* Data regarding the effect of UV irradiation of *C. musae* inoculated banana fruits is shown in Fig. 3. In general, effect of different wave lengths on growth of the pathogen was similar to those in vitro experiments. The highest lesion diameter (23.7 mm) by the inoculated fungus was recorded in control (light) treatment followed by 22.7 mm in control (dark). However, there was non-significant difference between the two control treatments. All the three UV irradiation treatments significantly reduced lesions diameters as compared to both the control treatments. The highest effect was due to irradiation of UV-C where fungal growth was completely checked. The lowest effect was recorded due to UV-A followed by UV-B irradiation. Earlier, Darras *et al.* (2012) reported that lesion diameters by *Botrytis cinerea* on florets of gerberas was reduced by 55% due UV-C irradiation. Storage rots of vegetables and fruits can be reduced by UV-C irradiation. Consequently self-life of the produce is increased (Wilson *et al.*, 1997). The control of storage rots by UV-C irradiation could be credited to its direct germicidal effect as well as its ability to stimulate defense responses in tissues of the host (Terry and Joyce, 2004; Darras *et al.*, 2012).The present work concludes that UV-C irradiation is highly effective in controlling the anthracnose disease of banana.

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