
*Journal of Biological Control*, 26 (3): 266–271, 2012


**Research Article**

# Efficacy of biological control agents for management of postharvest black rot pathogen *Aspergillus carbonarius* in grapes

**R. SENTHIL*, L. RAJENDRAN and K. PRABAKAR**

*Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India.*
** Corresponding author: E-mail: sensharuk008@gmail.com*

**ABSTRACT**: Grape berries were treated with different biological agents to test their efficacy in reducing post harvest incidence of black mould rot and minimize the loss. The agents *Pseudomonas, Bacillus, Trichoderma* and yeast isolates were individually screened against the black rot pathogen *Aspergillus carbonarius*. *B. subtilis* strains EPC-8 and EPCO-16 and *T. harzianum* (Th Co) showed high mycelial growth suppression of *A. carbonarius in vitro.* The effective biological control agents were tested in pre, post and combined inoculation studies against *A. carbonarius* in grape berries. In the pre inoculation, *B. subtilis* (EPC-8) showed 57.80 per cent reduction in the incidence of *A. carbonarius* followed by *T. harzianum* (Th Co) (48.43 percent). The same trend of effectiveness was also found in the post-inoculation and combined inoculation tests under storage conditions at room temperature in grape berries.

**KEY WORDS**: *Aspergillus carbonarius, Bacillus subtilis, Trichoderma harzianum.*

(Article chronicle: Received: 4-6--2012 Revised: 27-8-2012 Accepted: 11-9-2012)

## INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops in the world and India, 20 to 30% of fresh grapes are lost every year due to inadequate postharvest storage (El-Ghouth and Wilson, 1995). In cold storage grapes (–1°C), the main decay pathogens are *Botrytis cinerea* Pers., *Cladosporium herbarum* Link, *Alternaria alternata* Keissler, *Rhizopus stolonifer, Aspergillus carbonarius* (Bainier), *Aspergillus* sp. and *Penicillium expansum* Link. Further losses occur during grading, packing, transport and marketing as fresh produce or in the processed form (Ghosh, 1999). In general terms, post-harvest diseases destroy 10–30% of the total yield of crops and in some perishable crops especially in developing countries, losses are more than 30% (Agrios, 2005). Thakur and Saharan (2008) estimated that postharvest losses in grapes are about 39% of yield and 30% of value. Research has focused on developing alternative control methods against pre- and postharvest decay in grapes as well as in other crop. Biological control has emerged as an effective strategy to combat major postharvest decays of fruits (Janisiewicz and Korsten, 2002). Postharvest biocontrol is especially attractive because, harvested fruits are readily accessible to treatment with antagonists and many postharvest pathogens infect the fruits through wounds after harvest (Nunes *et al.,* 2001). Several isolates of bacteria and yeasts control postharvest pathogens on a range of perennial and annual crops (El-Ghaouth *et al.,* 1997). *Trichoderma harzianum* (Rifai) is an extremely versatile biocontrol agent suppressing diseases caused by a number of airborne plant pathogens, including anthracnose and grey mould in strawberry (Freeman *et al*., 2004). Sivakumar *et al.* (2000) reported that *T. harzianum* (TrH 40) is antagonistic against the postharvest pathogens *Gliocephalotrichum microchlamydosporum, Colletotrichum gloeosporioides* and *Botryodiplodia theobromae.* Against this background, the present study was carried out to evaluate the effect of bioagents as pre, combined, and post-inoculation for the management of black rot disease in grape berries.

## MATERIALS AND METHODS

### Isolation of *Aspergillus carbonarius*

Grape berries showing typical symptoms of disease were surface sterilized with 0.1% mercuric chloride for 1 min and washed three times in sterile distilled water. Sterile pieces of symptomatic grape berries were plated in sterile of potato dextrose agar (PDA) and incubated at room temperature (28 ±2°C) to isolate the *Aspergillus carbonarius*. Pure culture of the isolated fungus was obtained by single spore isolation technique (Riker and Riker, 1936) and maintained in PDA slants.

**Testing Koch's postulates**

Healthy grape berries were washed in running tap water, surface sterilized with 0.1 per cent mercuric chloride and washed thrice with sterile distilled water before inoculation of the pathogen. Injuries were made with a sterile needle up to a diameter of 5 mm and a disc of mycelium was immediately placed over it. The inoculated area was covered with moist cotton and the grapes were placed inside sterile perforated polythene bags (200 gauge) and sprayed with sterile distilled water to provide the required humidity and the berries were incubated at room temperature (28 ± 2°C) for 15 days (Franck *et al.,* 2005).

**Isolation and collection of biocontrol agents**

*Trichoderma harzianum* (Th Co) was isolated from soil samples collected from grapevine fields in Coimbatore, India. Five soil suspensions were prepared by adding 1.0 g of soil to 10 mL of sterile distilled water and shaking for 15 min and each suspension was serially diluted to $10^{-5}$, and 0.1 mL from the final dilution was spread on PDA medium and incubated at 28°C for 5 days. The isolated fungi were purified by single spore isolation technique (Riker and Riker, 1936) and maintained in PDA slants. The epiphytic yeast was isolated by shaking five grape berries in 10 mL of sterile distilled water for 1 h at 200 rpm on a rotary shaker (Peng and Sutton, 1991). The yeast solution was centrifuged at 5000 rpm for 10 min. The pellet was serially diluted to $10^{-5}$ and 1 mL of each dilution was spread on yeast extract peptone dextrose agar (YPDA) containing 20 g glucose, 10 g yeast extract, 10 g protease peptone and 15 g agar amended with 250 mg Penicillin G (to suppress growth of bacteria) in 1 L of distilled water. The Petri dishes were incubated at room temperature for 4 days and yeast colonies were transferred to YPDA plates to obtain pure cultures. Pure culture was stored at 4°C until used. The fungal antagonists *Trichoderma viride* (Tv1) and bacterial antagonists *Pseudomonas fluorescens* (Pf1) and *Bacillus subtilis* (EPCO-16 and EPC-8) were obtained from the Culture Collection Centre, Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India.

***In vitro* antagonistic activity**

*Trichoderma viride* (Tv1), *T. harzianum* (ThCo), *P. fluorescens* (Pf1), *B. subtilis* (EPC8 and EPCO16), *Candida albicans* (CY1 and CY2) and *Saccharomyces* sp. (SY1 and SY2) were tested for their efficacy by the dual-culture technique (Dennis and Webster, 1971) in PDA. The Petri dishes were incubated at room temperature (28±2°C) and the zone of inhibition was measured (Kishore *et al.*, 2005). Radial mycelial growth of the pathogen and the per cent reduction over the control was calculated using the formula: Inhibition over control (%) = C-T/C × 100; where, C- mycelial growth of pathogen in control and T- mycelial growth of pathogen in dual culture. Three replications were maintained for each antagonist.

**Preparation of inoculum of *B. subtilis* (EPC-8)**

The endophytic bacteria were grown in nutrient broth with constant shaking at 150 rpm for 48 h at room temperature (28 ± 2°C). The bacterial cells were harvested by centrifugation at 10,000 rpm for 15 min, and resuspended in phosphate buffer (0.01 M, pH 7.0). The concentration was adjusted using a spectro-photometer to approximately $10^8$ cfu/mL (OD595 = 0.3) and used as bacterial inoculum (Thompson, 1996)

**Preparation of inoculum of *T. harzianum* (Th Co)**

Nine mm disc of 7 days old culture of *T. harzianum* isolate was inoculated on PDA. The cultures were incubated for 7 days at room temperature (28 ± 2°C). 10 ml of sterile distilled water was added to the Petri dish to scrap the conidial mass. The conidial load was adjusted to $10^8$ cells per mL by serial dilution technique. A drop of wetting agent Tween 20 (polyoxyethylene sorbitan monolaurate) was added to every 250 mL of spore suspension.

**Effect of pre, post, and combined inoculation of biological agents on disease incidence**

Fully ripened Muscat grape berries were selected and individually treated with the bacterial cell ($8\times10^8$ CFU $mL^{-1}$) and *Trichoderma* spores ($3\times10^5$ CFU $mL^{-1}$) by dipping 100 berries in liquid culture containing the antagonist. Two days later, the berries were inoculated with a spore suspension ($3\times10^5$ CFU $mL^{-1}$) of *A. carbonarius* pathogen. In combined inoculation, the berries were first treated with biocontrol agents and then immediately treated with spore suspension of the pathogen. In post inoculation, grape berries were selected and inoculated with spore suspension of pathogen and two days later, the berries were treated with the respective antagonist. Six replications of each treatment using a completely randomized design were maintained in a sterile perforated polythene bag and incubated up to 10

days at room temperature. The per cent disease index (PDI) was calculated for each treatment as the percentage of berries showing disease symptoms in relation to the total number of berries in that treatment using the following empirical, disease grade: 0, bunch without rot; 1, 0–10% rotted berries; 2, 10–25%; 3, 25–50%; 4, 50–75% and 5, more than 75% rotted berries. The disease index was calculated using Mc Kinney's (1923) formula: PDI = sum of numerical ratings ×100/total number of bunches observed × maximum disease grade. In the combined inoculation tests and the post-inoculation tests the PDI was calculated in the same way.

**Statistical analysis**

Data were statistically analyzed using IRRISTAT (Version 92, developed by the International Rice Research Institute Biometrics Unit, The Philippines) and treatment means were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984). Analysis was done using the values in the table transformed by the arcsine method.

## RESULTS AND DISCUSSION

In the present study, *A. carbonarius* causing the black rot was isolated from grapes berries collected from different markets of Tamil Nadu. The pathogenicity was tested and the Koch's postulates fulfilled. Currently, biological control is considered a very promising alternative to synthetic fungicide in the control of postharvest decay of fruits and vegetables (Wisniewski and Wilson, 1992). The present study revealed that *B. subtilis* strain EPC-8 was the most effective, suppressing 88.7% of *A. carbonarius* mycelia growth (Table 1). *T. harzianum* strain (Th Co) was the next most effective, suppressing 82.3% of *A. carbonarius* mycelial growth. Several mechanisms may control this suppression, either directly inside the plant, by antibiosis against the pathogen (Sturz *et al.,* 1998) and by the competition for nutrients (Mari *et al.,* 1996), or indirectly by inducing a resistance response in the plant (M'Piga *et al.,* 1997). *B. subtilis* produces iturin, a powerful antifungal peptide (Gueldner *et al*., 1988), as well as gramicidin S (Edwards and Seddon, 2001). *T. harzianum* reduced Ochratoxins production but, had a greater effect on *A. carbonarius* growth (Valero *et al.,* 2006). Muthuraman and Sekar (1993) found that *T. viride* and *T. harzianum* inhibited the onion wilt pathogen, *Fusarium oxysporum* f. sp. *cepae in vitro.* In the current study, none of the yeast isolates was effective against *A. carbonarius* rot in grapes but, Zhang *et al.* (2005) reported that *Candida laurentii* was a potential biocontrol agent against postharvest gray mold rot *(B. cinerea)*, blue mold rot (*P.expansum*), and *Rhizopus* rot (*R. stolonifer)* respectively.

**Table 1. Efficacy of biological agents on *Aspergillus carbonarius* of grapes *in vitro***

| Biocontrol agent | *Aspergillus carbonarius** | |
|---|---|---|
| | Radial growth of pathogen (mm) | Inhibition over control (%) |
| *Trichoderma viride* (Tv1) | 80.8 (64.1)[d] | 10.1 |
| *Trichoderma harzianum* (Th Co) | 15.9 (23.5)[b] | 82.3 |
| *Pseudomonas fluorescens* (Pf1) | 85.2 (67.5)[de] | 5.3 |
| *Bacillus subtilis* (EPCO-16) | 32.7 (34.9)[c] | 63.7 |
| *Bacillus subtilis* (EPC-8) | 10.1 (18.6)[a] | 88.7 |
| *Candida albicans* (CY1) | 86.6 (68.5)[de] | 3.8 |
| *Candida albicans* (CY2) | 88.5 (70.34)[e] | 1.69 |
| *Saccharomyces* sp. (SY1) | 89.1 (70.88)[e] | 0.98 |
| *Saccharomyces* sp. (SY2) | 86.3 (68.38)[de] | 4.11 |
| Control | 90.0 (71.76)[e] | – |

* Mean of six replications. Values followed by the same letter are not significantly different at the 5% level by DMRT. Values in parentheses are arcsine transformed values.

In the present study, pre, post and combined treatment with *B. subtilis* (EPC-8) reduced the incidence of postharvest rot of grapes caused by *A. carbonarius*. Pre inoculated *B. subtilis* (EPC-8) reduced *A. carbonarius* by 57.80% followed by *T. harziamun* (Th Co) with 48.43% after 10 days at room temperature when compared to control (Table 2). The results of combined inoculation, *B. subtilis* (EPC-8) was treated first and then immediately inoculated with *A. carbonarius,* showed that the per cent reduction of incidence of *A. carbonarius* was 67.6% compared to control (Table 3). This was followed by treatments with *T. harziamun* (Th Co) (63.44%) and EPCo–16 (53.97%). Post-inoculation of *B. subtilis* (EPC-8) on grape berries was significantly effective, reducing the incidence of rot caused by *A. carbonarius* by 47.3%. This was followed by *T. harzianum* (Th Co) which also significantly reduced post harvest pathogen incidence (38.32%) compared to control (Table 4). *B. subtilis* is an antagonist against the major postharvest pathogens of stone fruits (Pusey *et al*., 1988), pome fruits (Wilson *et al.,* 1993) and citrus fruits (Smilanick and Denis-Arrue, 1992).

**Table 2. Effect of pre inoculation of antagonists on the incidence of *Aspergillus carbonarius* in grapes**

| Treatment | Percentage disease index (PDI)* Days after treatmentcontrol on | | | | | Mean | Reduction over 10th day (%) |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | | |
| *Bacillus subtilis* (EPC-8) | 3.66 (11.03)[a] | 8.14 (16.57)[a] | 12.59 (20.78)[a] | 15.56 (23.23)[a] | 22.22 (28.12)[a] | 14.63 | 57.80 |
| *Bacillus subtilis* (EPCO-16) | 5.87 (14.02)[b] | 10.74 (19.13)[b] | 16.29 (23.80)[b] | 21.48 (27.61)[b] | 28.29 (32.13)[b] | 16.53 | 46.28 |
| *Trichoderma harzianum* (Th Co) | 4.44 (12.16)[b] | 9.25 (17.71)[b] | 14.07 (22.03)[b] | 20.37 (26.83)[b] | 24.07 (29.38)[b] | 14.44 | 48.43 |
| Control | 8.14 (16.58)[c] | 17.04 (24.38)[c] | 28.96 (32.55)[c] | 36.37 (37.09)[c] | 52.66 (46.53)[c] | 28.63 | |

*Mean of six replications. Values followed by the same letter are not significantly different at the 5% level by DMRT. Values in parentheses are arcsine transformed values.

**Table 3. Effect of combined inoculation of antagonists and pathogen on the incidence of *Aspergillus carbonarius* disease in grapes**

| Treatment | Percentage disease index (PDI)* Days after treatmentcontrol on | | | | | Mean | Reduction over 10th day (%) |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | | |
| *Bacillus subtilis* (EPC-8) | 0.77 (5.04)[a] | 2.59 (9.26)[a] | 7.41 (15.80)[a] | 12.59 (20.78)[a] | 17.04 (24.38)[a] | 7.93 | 67.64 |
| *Bacillus subtilis* (EPCO-16) | 2.48 (9.06)[b] | 6.29 (14.53)[b] | 10.74 (19.13)[b] | 15.55 (23.22)[b] | 21.48 (27.61)[b] | 11.11 | 53.97 |
| *Trichoderma harzianum* (Th Co) | 1.48 (6.99)[b] | 5.19 (13.16)[b] | 10.37 (18.78)[b] | 14.07 (22.03)[b] | 19.25 (26.02)[b] | 10.07 | 63.44 |
| Control | 8.14 (16.58)[c] | 17.04 (24.38)[c] | 28.96 (32.55)[c] | 36.37 (37.09)[c] | 52.66 (46.53)[c] | 28.63 | |

*Mean of six replications. Values followed by the same letter are not significantly different at the 5% level by DMRT. Values in parentheses are arcsine transformed values.

**Table 4. Effect of post inoculation of antagonists on the incidence of *Aspergillus carbonarius* in grapes**

| Treatment | Percentage disease index (PDI)* Days after treatmentcontrol on | | | | | Mean | Reduction over 10th day (%) |
|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 10 | | |
| *Bacillus subtilis* (EPC-8) | 3.33 (10.51)a | 8.89 (17.34)a | 14.37 (22.27)a | 19.76 (26.12)a | 27.77 (31.79)[a] | 15.31 | 47.27 |
| *Bacillus subtilis* (EPCO-16) | 4.07 (11.64)[b] | 12.03 (20.29)[b] | 19.59 (26.27)[b] | 25.66 (30.43)[b] | 30.37 (33.44)[b] | 18.34 | 34.93 |
| *Trichoderma harzianum* (Th Co) | 5.56 (13.64)[b] | 11.26 (19.61)[b] | 15.96 (23.55)[b] | 21.92 (27.92)[b] | 32.48 (34.74)[b] | 17.43 | 38.32 |
| Control | 8.14 (16.58)[c] | 17.04 (24.38)[c] | 28.96 (32.55)[c] | 36.37 (37.09)[c] | 52.66 (46.53)[c] | 28.63 | – |

*Mean of six replications. Values followed by the same letter are not significantly different at the 5% level by DMRT. Values in parentheses are arcsine transformed values.

## REFERENCE


Agrios GN. 2005. *Plant Pathol*, Academic Press, New York., NY, USA. 556 pp.

Dennis C, Webster J. 1971. Antagonistic properties of species-group of *Trichoderma* I production of non-volatile antibiotics. *Transcends British Mycol Soc.* **57**: 25–39.

Edwards SG, Seddon B. 2001. Mode of antagonism of *Brevibacillus brevis* against *Botrytis cinerea in vitro*. *J Appl Microbiol.* **91**(4): 652–659.

El-Ghaouth A, Arul J, Wilson CL, Benhamou N. 1997. Biochemical and cytochemical aspects of the interactions of chitosan and *Botrytis cinerea* in bell pepper fruits. *Postharvest Biol Technol.* **12**: 183–194.

El-Ghouth A, Wilson CL. 1995. Biologically based techniques for the control of postharvest diseases. *Postharvest News Infor.* **6**: 5–11.

Franck J, Latorre BA, Torres R, Zoffoli JP. 2005. The effect of preharvest fungicide and postharvest sulfur dioxide use on postharvest decay of table grapes caused by *Penicillium expansum*. *Postharvest Biol Technol.* **37**: 20–30.

Freeman S, Minz D, Kolesnik I, Barbul O, Zveibil A, Maymon M, Nitzani Y, Kirshner B, Rav-David D, Bilu A, Dag A, Shafir S, Elad Y. 2004. *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* and survival in strawberry. *European J Plant Pathol.* **110**: 361–370.

Ghosh SP. 1999. Research preparedness for accelerated growth of horticulture in India. *J Appl Hortic.* **1**(1): 64–69.

Gomez KA, Gomez AA. 1984. *Statistical Procedures for Agricultural Research*, second edition, Wiley, New York, NY, USA. 198 pp.

Gueldner RC, Reilly CC, Pusey RL, Costello CE, Arrendale RF, Cox RH, Himmelsbach DS, Crumley FG, Cutler HG. 1988. Isolation and identification of iturins as antifungal peptides in biological control of peach brown rot with *Bacillus subtilis*. *J Agric Food Chem.* **36**: 366– 370.

Janisiewicz WJ, Korsten L. 2002. Biological control of postharvest diseases of fruit. *Annu Rev Phytopathol.* **40**: 411–441.

Kishore GK, Pande S, Podile AR. 2005. Biological control of collar rot disease with broad-spectrum antifungal bacteria associated with groundnut. *Can J Plant Pathol.* **51**: 123–132.

M'piga P, Bélanger RR, Paulitz TC, Benhamou N. 1997. Increased resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. *Physiol Mol Plant Pathol.* **50**: 301–320.

Mari M, Guizzardi M, Pratella GC. 1996. Biological control of gray mold in pears by antagonistic bacteria. *Biol Control* **7**: 30–37.

Mc Kinney HH. 1923. A new system of grading of plant disease. *J Agric Res.* **26**: 195–218.

Muthuraman G, Sekar R. 1993. Biological control of onion basal rot disease by antagonistic *Trichoderma* sp. *J Bio Control.* **7**: 114 -120.

Nunes C, Usall J, Teixido N, Vinas I. 2001. Biological control of postharvest pear diseases using a bacterium *Pantoea agglomerans* CPA-2. *Int J Food Microbiol.* **70:** 53–61.

Peng G, Sutton JC. 1991. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. *Can J Plant Pathol.* **13**: 247–257.

Pusey PL, Hotchkiss MW, Dulmase HT, Baumgardner RA, Zehr EI, Reilly CL, Wilson CL. 1988. Pilot tests for commercial production and application of *Bacillus subtilis* B-3 for postharvest control of peach brown rot. *Plant Dis.* **72**: 622–626.

Riker AJ, Riker RS. 1936. *Introduction to Research of Plant Disease*. Johns. Swiltc. MC. New York. NY, USA. 177 pp.

Sivakumar D, Wilson RS, Wijeratnam R, Wijesundera RLC, Marikar FMT, Abeyesekere M. 2000. Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of rambutan (*Nephelium lappaceum*). *Phytoparasitica* **28**: 3.

Smilanick JJ, Denis-Arrue R. 1992. Control of green mould lemons with *Pseudomonas* species. *Plant Dis.* **76**: 481.

Sturz AV, Christie BR, Matheson BG. 1998. Association of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. *Can J Plant Pathol.* **44**: 162–167.

Thakur AK, Saharan, VK. 2008. Effectiveness of shrink wrap on quality and shelf life of apple. *J Food Sci Tech.* **46**(5): 440-445.

Thompson DC. 1996. Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. *Plant Dis.* **80**: 856–862.

Valero A, Farre JR, Sanchis V, Ramos AJ, Sonia Marin S. 2006. Effects of fungal interaction on ochratoxin A production by *A. carbonarius* at different temperatures and water activity. *Int J Food Microbiol.* **110**: 160-164.

Wilson CL, Wisniewski ME, Droby S, Chalutz E. 1993. A selection strategy for microbial antagonists to control postharvest diseases of fruits and vegetables. *Sci Hortic.* **53**: 183–189.

Wisniewski ME, Wilson CL. 1992. Biological control of postharvest diseases of fruits and vegetables: recent advances. *Hort Science* **27**: 94–98.

Zhang H, Zheng X, Fu C, Xi Y. 2005. Postharvest biological control of gray mold rot of pepper with *Cryptococcus laurentii*. *Postharvest Biol Technol.* **35**: 79–86.


*Journal of Biological Control*, 26 (3): 272–273, 2012


**Research Article**

# Occurrence of Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) on cotton in India and record of associated parasitoids

**A. N. SHYLESHA* and SUNIL JOSHI**

*National Bureau of Agriculturally Important Insects, H.A. Farm Post, Bellary Road, Hebbal, Bangalore 560 027, Karnataka, India*
** Corresponding author: E-mail: anshylesha@gmail.com*

**ABSTRACT**: The invasive mealybug *Phenacoccus madeirensis* Green was recorded from cultivated cotton crop near Bandipur National park, Karnataka. Although the mealybug was recorded previously on *Cestrum nocturnum* during the current year, severe infestation was recorded for the first time on cotton crop. The parasitoid fauna recorded from *P. madeirensis* included predominantly *Anagyrus quadrii, Anagyrus loecki* and *Alotropa* spp.

**KEY WORDS**: *Phenacoccus madeirensis*, parasitoids, *Anagyrus qadrii, Anagyrus loecki*

(Article chronicle: Received: 3-9-2012 Revised: 10-9-2012 Accepted: 14-9-2012)

## INTRODUCTION

Globalization of Indian commodity market and concomitant increase in international travel has led to the influx of exotic invasive species. Often species introduced without their natural enemies from the new World to old World and vice versa become invasive and cause loss of biodiversity, modify the habitat and cause extensive environmental and economic harm (Fish *et al.,* 2010). In the recent years, several species of economically important pests have been introduced to India *viz*., the papaya mealybug, *Paracoccus marginatus* Williams and Granara De Willink, eucalyptus gall wasp *Leptocybe invasa* Fisher and La Salle, and the erythrina gall wasp, *Quadrastichus erythrinae* Kim, causing huge losses to the growers. Several sucking pests are waiting to enter India *viz.,* the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, Jack Beardsley mealybug, *Pseudococcus jackbeardsleyi* Gimpel and Millerthe, Madeira mealybug, *Phenacoccus madeirensis* Green and giant whitefly, *Aleurodicus dugesii* (Muniappan *et al.,* 2011). Recent surveys for invasives in South India undertaken by NBAII, Bangalore has confirmed the occurrence of Madeira mealybug on *Cestrum nocturnum* (Solanaceae) (Night Queen Flower or Night blooming jasmine) (NBAII News letter 2011) and consequently frequent occurrence on several other crops.

The *P. madeirensis* is of neotropical origin and widespread in South America. It had established in Micronesia and Africa. Williams (2004) recorded it in Pakistan in 1997. Quarantine interceptions in 2010 indicated its occurrence in Philippines, Vietnam and Thailand, Taiwan in 2006 (Muniappan *et al.,* 2011). It is a polyphagous species known to feed on 44 plant families including fruits, vegetables and ornamental crops. As per the Government of India, Ministry of Agriculture Notification dated July 2007 – called the Plant Quarantine (Regulation of Import into India) First Amendment, Order, 2007 *P. madeirensis* is listed under the quarantine pest and caution has been given for the importation from 45 countries enlisted in the order. Among the list of 85 countries enlisted in the distribution map, incidence in India has not been recorded (CABI, 2000). This is the first report of occurrence of *P. madeirensis* from India.

*Phenacoccus madeirensis* was first described from specimens collected on the Madeira Island (Green, 1923). It is often misidentified as the Mexican mealybug, *Phenacoccus gossypii* Townsend & Cockerell. A review of *P. gossypii* and its related species by Williams (1987) has clarified the taxonomic confusions between *P. madeirensis* and *P. gossypii*. The two closely related species differ in that *P. gossypii* possesses numerous multilocular pores on the median dorsal areas of the thorax, which is a morphological characteristic absent in *P. madeirensis*.

**Field Characters:** Body oval; gray coloured; somewhat flattened dorsoventally; legs red; covered by thin, white, mealy wax, with dark dorsosubmedial bare

spots on intersegmental areas of thorax and abdomen, these areas forming 1 pair of dark longitudinal lines on dorsum; ovisac covering entire dorsum; with 18 pairs of lateral wax filaments, posterior pairs longest, about or less of length of the body. Predominantly occurring on foliage of host plants. Specimens in alcohol with 1 pair dorsosubmedial dark lines on thorax and abdomen. Surface of lateral filaments rough (Plate 1, 2 and 3).

**Validation characters:** Cerarii-like aggregations on anterior abdomen, thorax, and head, but not on dorsomedial areas of abdominal segments VI and VII; multilocular pores on dorsum of abdomen, absent from thorax and head; dorsal oral-collar tubular ducts scattered over surface; quinquelocular pores abundant on ventral surface; denticle on claw; antennae 9-segmented (Fig. 1).

**Comparison:** *P. madeirensis* is very similar to *P. gossypii* and *P. franseriae* Micro by having dorsal multilocular pores and dorsal oral-collar tubular ducts; cerarii on medial or mediolateral areas of dorsum; numerous quinquelocular pores; mushroom-shaped circulus. *P. madeirensis* differs by having cerarii-like aggregations on thorax and head only and by having dorsal multilocular disk pores absent from the mediolateral areas of the thorax. While *P. gossypii* has dorsal mediolateral multilocular disk pores on the thorax, and *P. franseriae* has dorsomedial cerarii on abdominal segments VI and VII.

### Incidence in cotton

Cotton cultivated between Bandipur and Gundlupet on Mysore to Udagamandalam high way was surveyed for the incidence of mealybugs. The cotton crop in an area of about 7.5 ha was severely affected by mealybug. Samples were collected from the fields along with the parasitoids and predatory fauna associated with it. On examination of the identity of the mealybug it was confirmed as *P. madeirensis*. The incidence was to the tune of 90-100 percent of the cotton plants in the entire area. Leaf, stem, flower bases and bolls were highly infested by *P. madeirensis* mealybug and boll opening was improper in many plants. The following species of parasitoids were recorded in the field as well as from the samples collected and kept for incubation in laboratory *Allotropa* sp. *Anagyrus* sp. *Anagyrus qadrii* (Hayat, Alam & Agarwal, 1975*), Anagyrus loecki* Noyes & Menezes and hyper parasitoids, *Prochiloneurus aegyptiacus* (Mercet) and *P. javanicus* Ferriere. Among them the population of *A. qadrii* was more compared "to *Anagyrus* sp. and was found to parasitize efficiently (Table 1).

**Table 1. Emergence of parasitoids from *Phenacoccus madeirensis* infested cotton twigs**

| Sl. No. | Parasitoids emerged | Average number of parasitoids/ twig (5cm) |
|---|---|---|
| 1 | *Allotropa* sp | 5 |
| 2 | *Anagyrus* sp. | 2 |
| 3 | *Anagyrus qadrii* | 13 |
| 4 | *Anagyrus loecki* | 8 |
| 5 | *Prochiloneurus aegyptiacus* | 3 |
| 6 | *P. javanicus* | 2 |

N = 5 twigs

## ACKNOWLEDGEMENT

The authors are thankful to Dr. J. Poorani, Principal Scientist, Biosystematics Division of NBAII for the identification of the parasitoids.

## REFERENCES

CABI. 2000. *Phenacoccus madeirensis* Green. Distribution Maps of Pests Map No. 607. CAB International, UK 4 pp.

Fish J, Chiche Y, Day R, Efa N, Witt A, Fessehaie R, Johnson KDG, Gumisizira G, Nkandu B. 2010. Mainstreaming gender into prevention and management of invasive species. Global Invasive Species Programme, 63 p. www.gisp.org

Green EE. 1923. Observations on the Coccoidae of the Madeira Islands. *Bull Ent Res*. **14:** 87–97.

Muniappan R, Shepard BM, Watson GW, Carner GR, Rauf A, Sartiami D, Hidayat P, Afun JVK, Goergen G, Ziaur Rahman AKM. 2009. New Records of Invasive Insects (Hemiptera: Sternorrhyncha) in Southeast Asia and West Africa. *J Agric Urban Ent*. **26**(4): 167–174.

Williams DJ. 2004. *Mealybugs of Southern Asia*. The Natural History Museum, Kuala Lumpur: Southdene SDN, BHD. 896 pp.

Williams DJ. 1987. *Phenacoccus gossypii* Townsend & Cockerell, *P. madeirensis* Green and some related mealybug species (Hemiptera: Pseudococcidae*). Bull Ent Res.* **77**: 335–356.