Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 8 (4): 144-151, 2009
ISSN 1812-5387
© 2009 Asian Network for Scientific Information

In vitro Assay of Factors Affecting the Growth of Pathogens Associated with Diseases on Dragon Fruit (*Hylocereus* spp.) in Peninsular Malaysia

[1]M. Masyahit, [1]K. Sijam, [2]Y. Awang and [3]M.G.M. Satar
[1]Department of Plant Protection,
[2]Department of Crop Science,
[3]Departement of Soil Science, Faculty of Agriculture, University of Putra Malaysia,
43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

Abstract: Knowing the unfavorable environment for the growth of a pathogen can be utilized as the basic information in developing appropriate strategies to prevent disease occurrence on dragon fruit. Several environmental factors including temperature, pH and salinity, as well as biotic factor including three antagonistic bacteria species, namely *Bukholderia cepacia*, *B. multivorans* and *Pseudomonas aeruginosa* against *Bipolaris* sp., *Colletotrichum gloeosporioides*, *Botryosphaeria* sp. and *Monilinia* sp., were investigated. Mycelial growth of all tested fungi was constantly inhibited by a temperature of 35°C, while a temperature of 25°C was quite suitable for their growth. A temperature of 30°C was favorable for the growth of *Colletotrichum gloeosporioides*. Under different pH condition, the growth of tested fungi was mostly inhibited by extreme pH of 4 and 10. The salinity assay showed that *Monilinia* sp. was not affected by all treatments among tested fungi. Only concentration 100 ppm could reduce the growth of *Bipolaris* sp., though its inhibition statistically affected on 4 and 6 Days after Incubation (DAI). Meanwhile, the *in vitro* examination of antagonistic bacteria resulted in *Bukholderia multivorans* which was highly effective in inhibiting the growth of examined fungi, except *Monilinia* sp., which was more significantly influenced by *B. multivorans* and *B. cepacia*. The proper combination of environmental modification may be useful for the growth of crop in the field as well as the storage life of the fruit at postharvest preservation.

Key words: Temperature, pH, salinity, antagonistic bacteria, dragon fruit

INTRODUCTION

Dragon fruit (*Hylocereus* spp.) is a vine climbing cactus species which originated from South America (Crane and Balerdi, 2005). This crop is characterized by a skin covered with dragon-like scales. It was firstly introduced in large scale into Malaysia about two decades ago by Golden Hope Company locating at Sungai Wangi Estate (Perak). Furthermore, at the beginning of 1999, the commercial cultivations were then developed in Kluang (Johor), Kuala Pilah (Negeri Sembilan) and Sitiawan (Perak) (Halimi and Satar, 2007).

Many records of diseases on dragon fruit have been documented from several white-fleshed and yellow species-producing countries. Some earlier studies have successfully recognized a number of fungal species causing various diseases, such as *Alternaria* sp., *Ascochyta* sp., *Aspergillus* sp., *Bipolaris cactivora*, *Botryosphaeria dothidea*, *Capnodium* sp., *Colletotrichum gloeosporioides*, *Dothiorella* sp., *Fusarium* sp., *Gloeosporium agaves*, *Macssonina agaves*, *Phytopthora* sp. and *Sphaceloma* sp. (FAO, 2004; Sijam *et al*., 2008; Le Bellec *et al*., 2006; Palmateer *et al*., 2007; Paull, 2007; Taba *et al*., 2006, 2007; Valencia-Botin *et al*., 2003; Wang and Lin, 2005). In Peninsular Malaysia, several pathogenic fungi on this crop, such as *Bipolaris* sp., *Botryosphaeria* sp., *C. gloeosporoides* and *Monilinia* sp., causing fruit and stem end rot, brown spot, anthracnose and fruit brown rot are found on this crop, respectively (Masyahit *et al*., 2008, 2009).

Knowledge of the basic biology of the pathogen, such as germination and sporulation in relation to environmental factors, is useful in the development of a more sustainable strategies of disease management (Xu *et al*., 2001). It is necessary to understand the precise environmental conditions for infection and disease development in order to determine the appropriate time of fungicide application and perhaps the implementation of

Corresponding Author: Masanto Masyahit, Department of Plant Protection, Faculty of Agriculture, University of Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

alternative disease control measures (Percich *et al*., 1997). This study assessed the environmental factors as well as antagonistic bacteria against isolated fungal pathogens of dragon fruit under *in vitro* conditions.

MATERIALS AND METHODS

The experiments were carried out in the Microbiology Laboratory, Department of Plant Protection, Faculty of Agriculture, University Putra Malaysia, during February-March 2009. Fungal isolates used in this experiment were sub-cultured on PDA (Oxoid Ltd., Basingtoke, Hampshire; England) from isolate collection of our two earlier studies (Table 1). Some environmental factors including temperature, pH and salinity and three antagonistic bacteria species, namely *Bukholderia cepacia*, *B. multivorans* and *Pseudomonas aeruginosa* against those pathogenic fungi were investigated.

Effect of temperature: Fungal isolates were cultured on PDA plates and then incubated at 4 different temperatures, 20, 25, 30, or 35°C for 10 days according to a slight modification of the method developed by Baird (2004). Fungal growth was monitored by measuring the mycelial diameter using a digital caliper (Digimatic Caliper; Mitutoyo Corporation, Japan) every two days. The treatments were replicated three times.

Effect of pH: Fungal isolates were cultured on PDA. pH had been adjusted to 4, 5.5, 7, 8.5 or 10. The pH was adjusted by dropping 1 N HCl for decreasing pH and 1 N NaOH for increasing pH into autoclaved-PDA media before cooling according to the method developed by Fayzalla *et al.* (2008). pH was determined using Delta 320 pH meter (Mettler, Toledo). Fungal growth was monitored by measuring the mycelial diameter using digital caliper every two days. The treatment of this experiment was replicated three times.

Effect of salinity: Fungal isolates were cultured on PDA plates for 10 days with five different concentrations of salinity, i.e., 0, 1, 10, 100, 1000 mg L^{-1}, which were adjusted by adding NaCl (Sharlau Chemie S.A., Barcelona, Spain) before autoclaving following the procedure developed by Al-Rokibah *et al.* (1998) with a little modification. Fungal growth was monitored by measuring the mycelial diameter using digital caliper every two days. Treatments were replicated three times.

Table 1: Fungi isolated from diseased dragon fruit used in this experiment

Fungi	Diseases	References
Bipolaris sp.	Fruit soft rot and stem end rot	Masyahit *et al.* (2008)
Colletorichum gloeosporioides	Anthracnose	Masyahit *et al.* (2009)
Botryosphaeria sp.	Brown spot	Masyahit *et al.* (2008)
Monilinia sp.	Fruit brown rot	Masyahit *et al.* (2008)

Effect of antagonistic bacteria: This experiment was conducted with dual culture procedure following method developed by Sijam and Dikin (2005) by culturing both fungal isolate and antagonistic bacterium in PDA plates for 10 days at room temperature. Antagonistic bacteria, i.e., *Bukholderia cepacia*, *B. multivorans* and *Pseudomonas aeruginosa*, were sub-cultured on NA (Oxoid Ltd., Basingtoke, Hampshire; England) from collection of Microbiology Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Putra Malaysia. A plug of fungal isolate (4 mm in diameter) was placed 2 cm from the edge of the 9 cm plate; while the antagonistic bacterium was line-streaked on 2 cm from other edge of the same plate. Fungal growth was monitored by measuring both the mycelial radial toward the margin of plate (considered as Ro) and that straight the bacterial streak (considered as Rt) using digital caliper every 2 days. The treatment of this experiment was replicated three times. Zone inhibition of antagonistic bacteria against fungal isolates was calculated with this following formula:

$$PI = \frac{Ro - Rt}{Ro} \times 100\%$$

PI = Percentage of inhibition (%)
Ro = Mycelial growth of fungi toward margin plate (mm)
Rt = Mycelial growth of fungi toward the bacterial streak (mm)

Statistical analysis: These *in vitro* experiments were employed with Completely Randomized Design (CRD). Following the statistical procedures developed by Gomez and Gomez (1984), data were analyzed with ANOVA under Duncan Multiple Range Test (DMRT) using SAS® System for Windows V8 software (SAS Institute Cary, North California; USA).

RESULTS AND DISCUSSION

Effect of temperature: Overall, test of temperature treatment showed that the mycelial growth of the fungi under test was constantly inhibited by a temperature of 35°C; while temperature 25°C was quite suitable for their growth. A temperature of 30°C was favorable for the growth of *C. gloeosporioides*. The mycelial growth of *Bipolaris* sp., *Botryosphaeria* sp. and *Monilinia* sp., was more encouraged by temperature 20 and 25°C for

10 days incubation (Fig. 1a, c and d) while temperatures range of 25-30°C was optimum for the growth of *C. gloeosporioides* in this study (Fig. 1b).

Effect of pH: The *in vitro* assay of pH effect generally revealed that the growth of tested fungi was mostly inhibited by extreme pH, i.e., pH 4 and pH 10. The inhibition of pH 4 was effective until last incubation, except for *Monilinia* sp. which was only influenced at 2 Days after Incubation (DAI); whilst pH 10 was significant for 6 days incubation. At 6 days after incubation, growth of the two fungal species, *Bipolaris* sp. and *Botryosphaeria* sp., were more significantly repressed than others. In the meantime, pH values at range 5.5-8.5 were less effective against the growth of tested fungi. The effect of pH treatment of 5.5-10 in range decreased after the fourth observation (8 DAI) (Fig. 2a-d).

Effect of salinity: The findings of salinity assay described that *Monilinia* sp., was not affected by all treatments among tested fungi. For this fungus, treatments were only significant at 0.05 level in inhibiting its growth at first observation and it then could reach optimum growth only at 4 DAI. The significant effect of inhibition against *C. gloeosporioides* was only shown until 6 days incubation, while those entire treatments had highly significant different effects on *Bipolaris* sp., at 4 DAI on the 0.01 level. All salinity concentrations did not significantly inhibit the growth of *Botryosphaeria* sp. Only concentration 100 ppm could reduce the growth of *Bipolaris* sp., though its inhibition statistically affected on 4 and 6 DAI (Fig. 3a-d).

Effect of antagonistic bacteria: The results of antagonistic bacteria test highlighted that *Bukholderia multivorans* was highly effective in inhibiting the growth of examined fungi, except *Monilinia* sp., which was more significantly influenced by *B. cepacia*. It was found that *Pseudomonas aeruginosa* was only more effective at 4, 6 and 10 DAI against *Bipolaris* sp., rather than others. The ability of tested bacteria in inhibiting the growth of *Bipolaris* sp., (Fig. 4a) and *C. gloeosporioides* (Fig. 4b) fungi was mostly optimum at 8 DAI and fluctuated against *Botryosphaeria* sp., with the peak at last incubation (Fig. 4c). Meanwhile, only *B. multivorans* gradually increased in inhibiting *Monilinia* sp. with the optimum at 8 DAI; while the inhibition percentage of others was decreased until the last incubation (Fig. 4d).

In general, the growth of tested fungal pathogenic in this study was significantly inhibited by the extreme (minimum and maximum) level of treatments, except for salinity treatment. Meanwhile, three antagonistic bacteria used could increasingly restrict the mycelial growth of tested pathogen, excluding against *Monilinia* sp. These findings, however, were unlike those examining other species of the same fungi on different crops. Maximum proportional germination of *Bipolaris sorokiniana* causing disease complex on *Poa pratensis* and *Agrostis palustris* was greatest at 25 and 30°C, respectively, but it failed to germinate at 35°C (Hodges 1975); while Barba *et al.* (2002) observed that the sporulation of *B. sorokiniana* on barley seed reached its maximum at 19.3°C.

Some slight different ranges of the most favorable temperatures were also found on other *Botryosphaeria*

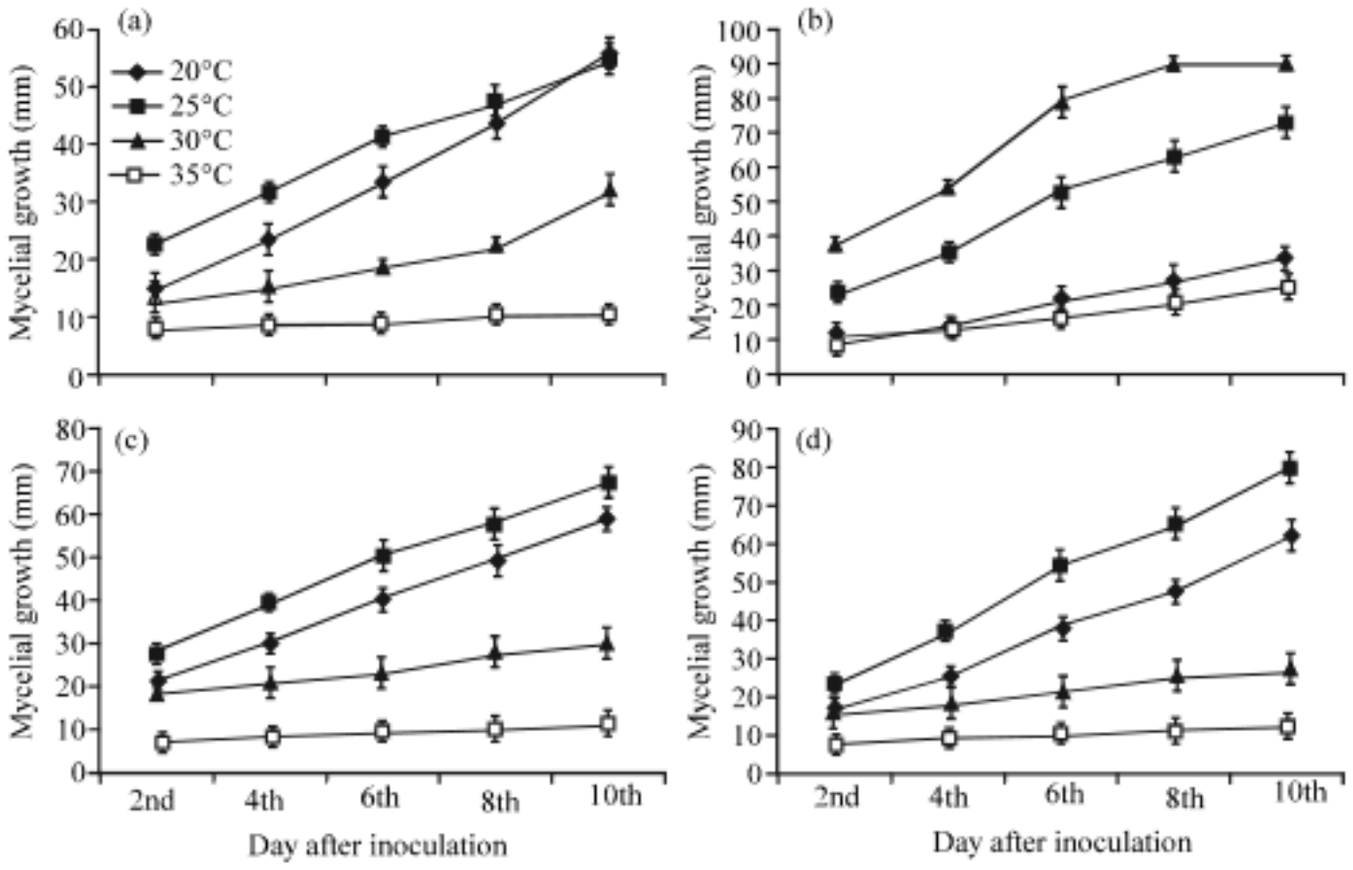

Fig. 1: The effect of temperature against (a) *Bipolaris* sp. (b) *Colletotrichum gloeosporioides* (c) *Botryosphaeria* sp. and (d) *Monilinia* sp., under *in vitro* condition

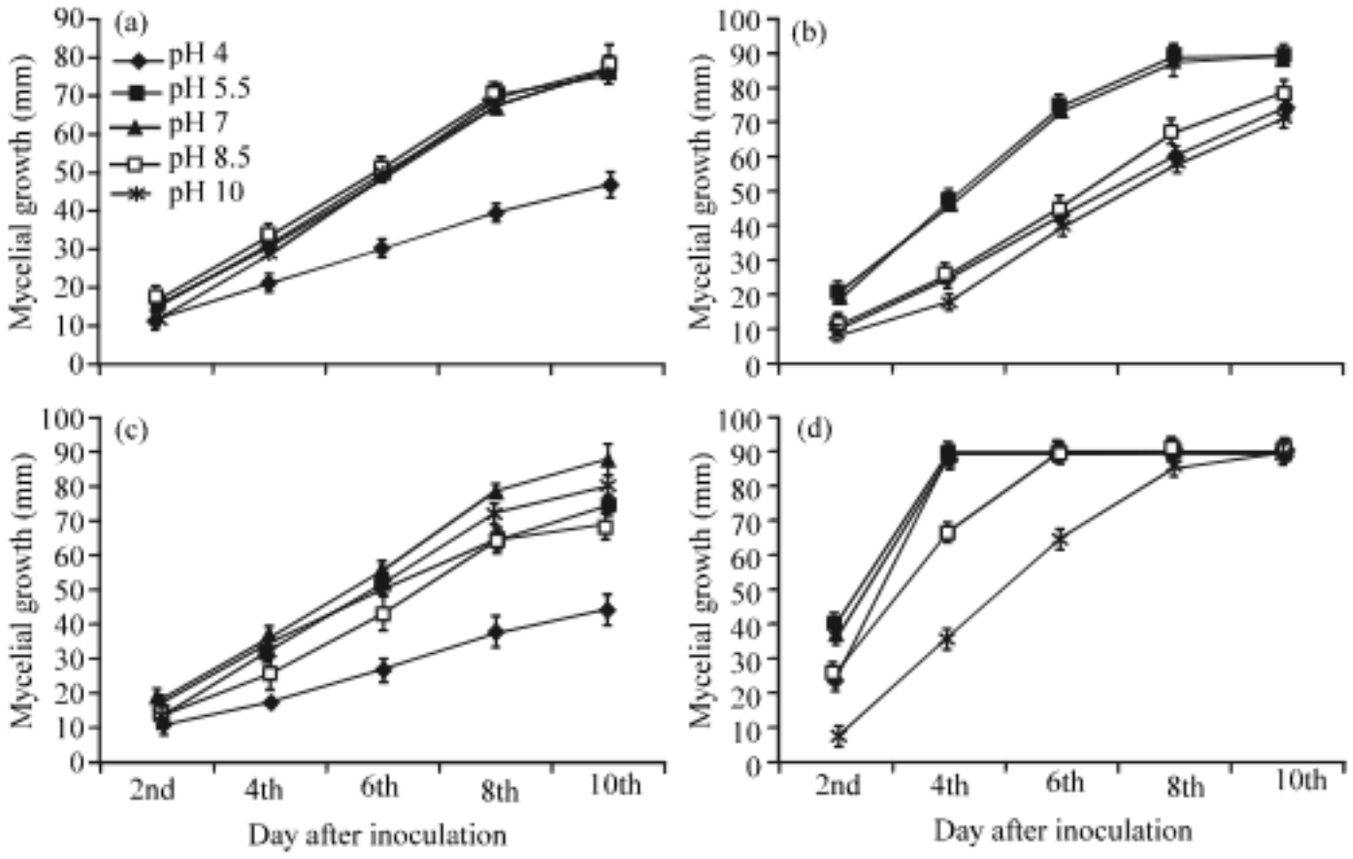

Fig. 2: The effect of pH level against (a) *Bipolaris* sp. (b) *Colletotrichum gloeosporioides* (c) *Botryosphaeria* sp. and (d) *Monilinia* sp., under *in vitro* condition

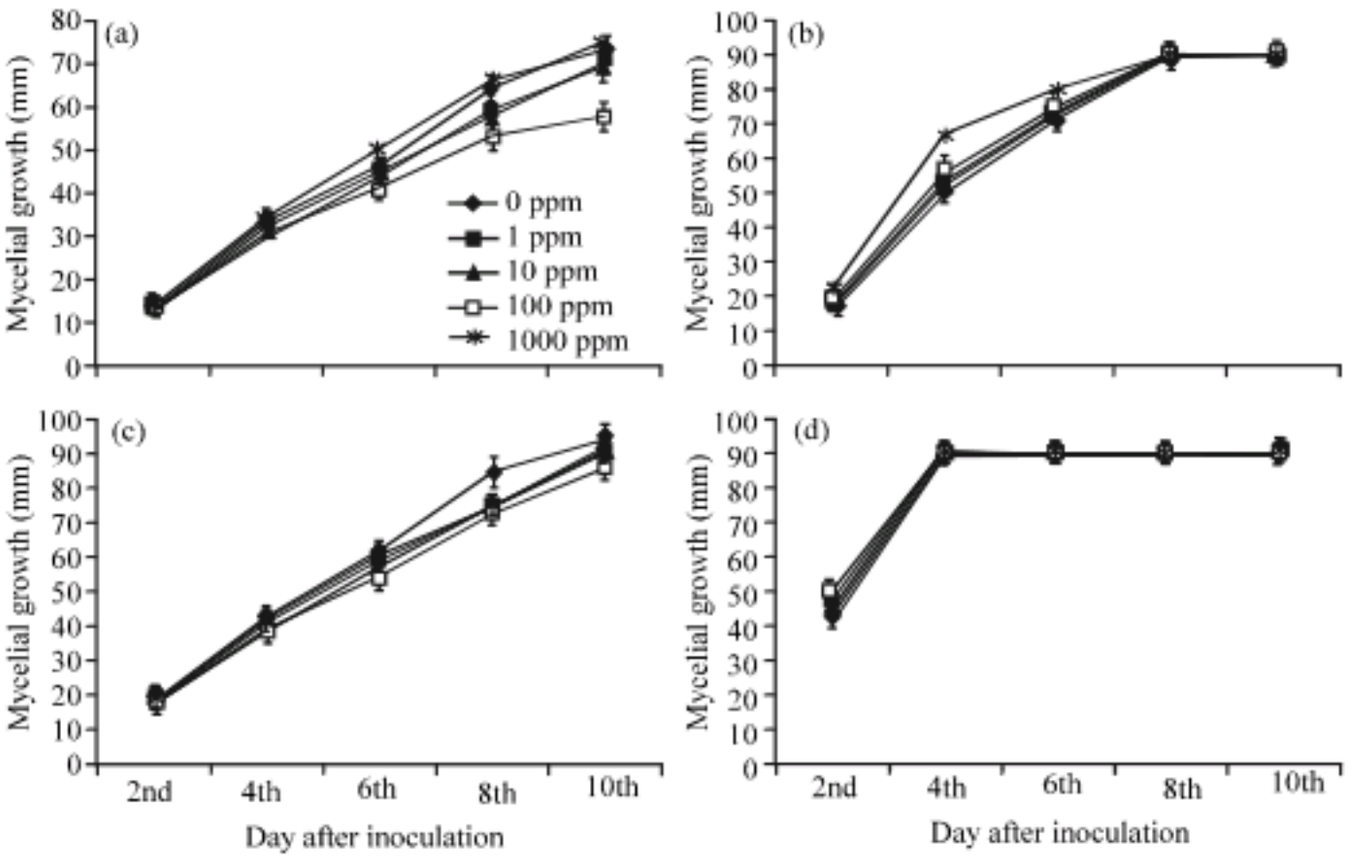

Fig. 3: The effect of salinity against (a) *Bipolaris* sp. (b) *Colletotrichum gloeosporioides* (c) *Botryosphaeria* sp. and (d) *Monilinia* sp., under *in vitro* condition

species. Pycnidial formation of *Botryosphaeria ribis* was induced either under continuous illumination at 21°C favoring development of uniloculate pycnidia or in alternating light (12-27°C) and darkness (12-21°C) enhancing formation of multiloculate pycnidia (Smith and Fergus, 1971).

Different optimum temperatures were even observed within *Monilinia* species by some authors on their previous studies. The rate of *M. fructicola* conidial growth on fresh stone fruits in incubators were maximum at 15°C and minimum at 25°C (Phillips, 1984); whereas like this current study, the conidia of *M. fructigena* causing brown rot disease on apple and pear fruit in UK had already germinated only 2 h after seeding on the plates at 20 and 25°C, about 48 and 55%, respectively and then declined at 30°C (Xu *et al*., 2001).

Meanwhile, temperature range of 25-30°C for optimum growth of *C. gloeosporioides* obtained in this study was slightly similar to those observed on earlier experiments on same fungus infecting other crops. Those works

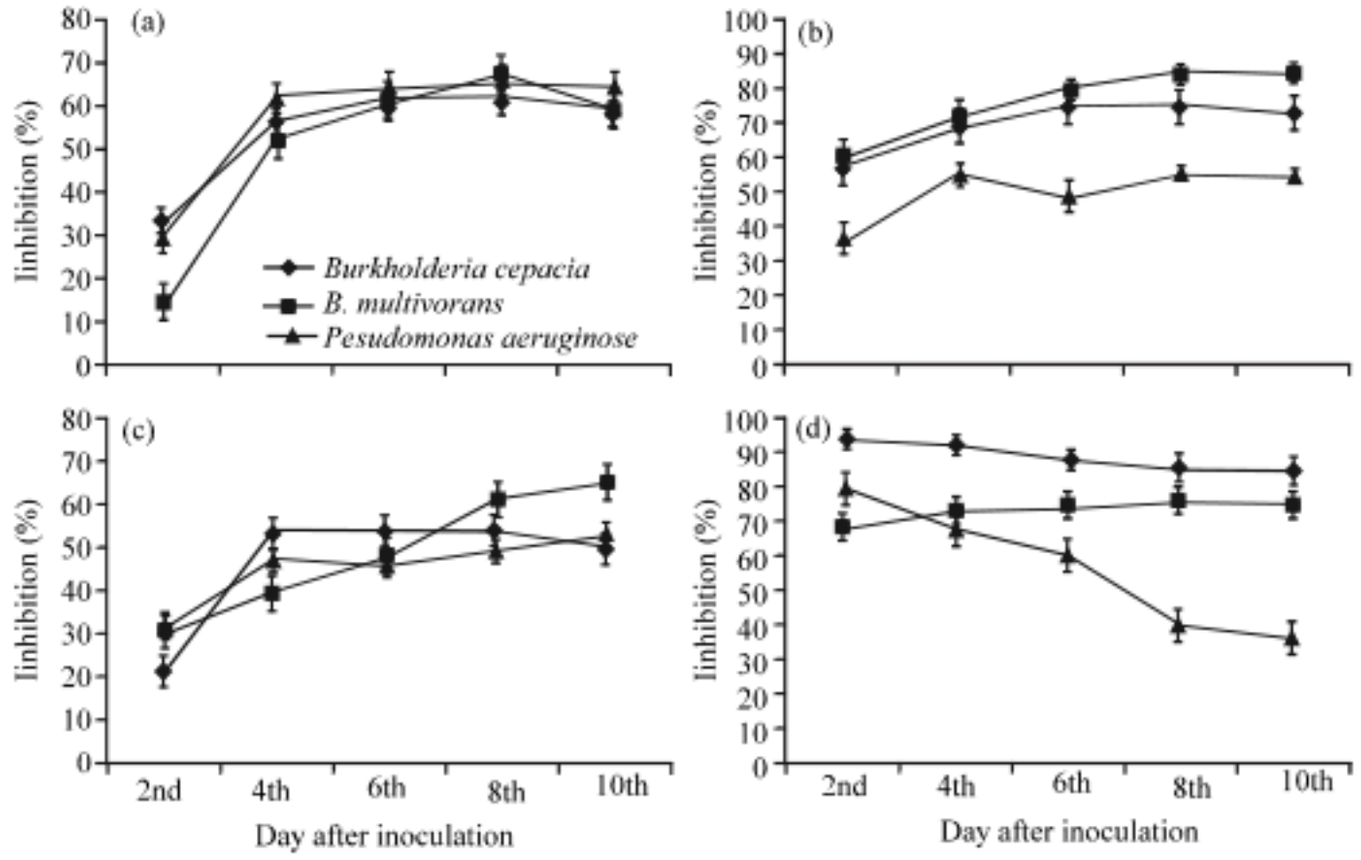

Fig. 4: The effect of antagonistic bacteria against (a) *Bipolaris* sp. (b) *Colletotrichum gloeosporioides* (b) *Botryosphaeria* sp. and (d) *Monilinia* sp., under *in vitro* condition

reported that a temperature range of 20-30°C as the optimum temperature for the lesion development on rubber leaves (Wastie, 1972), *in vitro* disease development on *Stylosanthes* sp. (Irwin *et al*., 1984) as well as germination and production of conidia (Fitzell and Peak, 1984), growth and sporulation (Davis *et al*., 1987), appressoria production (Estrada *et al*., 2000) and infection development (Akem, 2006) of *C. gloeosporioides* on mango.

The alkaline pH environment for *Bipolaris* sp., tested in this recent experiment was supported by other earlier study. Bischoff and Garraway (1987) assumed that accumulation of ammonium and increasing pH on solid media could augment the production of NADP-glutamate dehydrogenase and NAD-glutamate dehydrogenase in liquid culture of *Bipolaris maydis* which might be associated with either the presence of mycelium or the presence of mycelium with conidia.

The optimum pH (pH 5.5) for *C. gloeosporioides* found in this study (Fig. 1b) was parallel with a number of earlier researches. Tandon and Chandra (1962) reported that pH range of 4.0-6.5 was optimum condition for growth and sporulation of *C. gloeosporioides* and then Maccheroni *et al*. (2004) studied that acidic pH (pH 5.0) was favorable condition for pectin secretion as the indication of polygalacturonase activity in pathogenic *C. gloeosporioides*.

The non significant inhibitions of given salinity levels against growth of tested fungi in this trial are in agreement with some results obtained by earlier studies. The *in vitro* assay conducted by Hopkins and McQuilken (2000) resulted in the fastest hyphal extension rates of *Pestalotiopsis sydowiana*, the causal agent of foliage, stem-base and roots diseases on hardy ornamental crops at commercial nurseries in the UK occurring on PDA osmotically adjusted with NaCl. In the field study, MacDonald (1982) observed that salinity stress treatments could enhance the development of root rot lesion on Chrysanthemum caused by *Phytophthora cryptogea* as high 70 and 88% of roots previously exposed to 0.1 and 0.2 M, respectively.

The findings of this current study, however, differed with the field investigation carried out by Elmer (2003), who studied the role of NaCl in suppression of crown and root rot disease on asparagus (*Asparagus officinalis* L.) caused by *F. oxysporum* or *F. proliferatum*. He discovered that the reduction in root lesions was significantly higher on roots which were directly exposed to NaCl (51% reduction) than on non-exposed roots (31% reduction).

This *in vitro* assay demonstrated that *B. multivorans* was the most efficacious biocontrol agent compared to others, excluding against *Monilinia* sp. Dikin *et al*. (2007) reported that the antimicrobial substances of this bacterium could restrict the growth of *Schizophyllum commune*, the causal pathogen of brown germ and seed rot disease on oil palm.

This current experiment discovered that *B. cepacia* had the maximum percent of inhibition (93.11%) at 2 DAI against *Monilinia* sp. Previously, Janisiewicz and Roitman (1988) discovered that *B. cepacia* could reduce *in vitro* growth and conidia germination of the stone fruit

pathogen, *M. fructicola* by producing pyrrolnitrin. This recent study was not in agreement with the earlier experiment conducted by Bosch *et al.* (1992), who obtained the minimum inhibition (23%) of *B. cepacia* (syn. *Pseudomonas cepacia*) against *M. fructicola* causing brown rot on peach.

Against *Bipolaris* sp., this experiment revealed that *B. cepacia* could decrease its radial growth by 61.8% of optimum inhibition level for 8 days incubation. This was in range of inhibition level previously observed by (Jayaswal *et al*., 1993) on produced diffusible antifungal compounds against *Helminthosporium maydis* (syn. *Bipolaris maydis*) with around 58-75% for 3-5 days incubation but lower than against *H. turcicum* (syn. *Bipolaris turcicum*) with 76-83%..

On the other hand, the finding on *B. cepacia* against *C. gloeosporiodes* reported in this study showing 75.65% as optimum inhibition was higher than its inhibition on mycelial growth of the same pathogen isolated from anthracnose disease on papaya in post harvest storage (around 74.13%) (Rahman *et al*., 2007). In more recent study, Kadir *et al*. (2008) reported that higher dilution (1:8) of the antifungal substances in crude supernatant of *B. cepacia* was able to reduce both the mycelial growth and spore germination of *C. gloeosporioides* from similar isolate around 41 and 100%, respectively.

Differed with earlier study of *B. cepacia* effect on *Diplodia maydis* (syn. *Botryosphaeria maydis*) resulting in 42% of maximum inhibition level by the diffusible antifungal compounds for 5 days incubation (Jayaswal *et al*., 1993), this current study obtained higher inhibition level of *B. cepacia* on *Botryosphaeria* sp., namely 53.99% at 4 DAI. Jayaswal *et al*. (1993), however, showed about 75% optimum inhibition by the volatile compounds of this bacterium on *D. maydis* during 5 days incubation.

On other pathogenic fungi, Rahman *et al*. (2007) highlighted 68.45% of *P. aeruginosa* inhibition on the growth of *C. gloeosporioides* causing anthracnose disease on post harvest storage of papaya (*Carica papaya* L.) in Malaysia during *in vitro* screening on PDA medium. It was higher than that found on same fungi in this study, namely 55.41% of optimum inhibition at 4 DAI.

The absolute inhibitions were reached when the produced diffusible substances and bacterial suspension of *P. aeruginosa* were examined on mycelial growth of *C. gloeosporioides* isolate from papaya (Rahman *et al*., 2007). On the contrary, the lower effects were provided by its volatile substances on radial growth and spore germination, by its suspension (14.36 and 3.7%, respectively) and by its filter-sterilized culture filtrate on those two parameters (16.91 and 1.31%, respectively) of similar fungal isolate (Rahman *et al*., 2007).

CONCLUSION

In conclusion, the mycelial growth of tested fungi was highly affected by extreme condition of temperature (35°C) and pH (pH 4.0). Only concentration of 100 ppm could significantly inhibit the radial growth of certain given fungi; while among employed antagonistic bacteria, *Burkholderia multivorans* was the most effective in restricting the growth of the test fungi followed by *B. cepacia* and *Pseudomonas aeruginosa* *in vitro*. The proper combination of environmental modification may be useful for the growth of crop in the field as well as the storage life of the fruit at post harvest preservation.

ACKNOWLEDGMENTS

This study was conducted as the part of research project funded by the Fundamental Research Grant Scheme (FRGS) project No. (01-01-07-094FR) (2008/2009) under the Ministry of Higher Education, Malaysia. The authors also acknowledge Dr. Yahya Awang for suggestions in experimental design and model of statistical analysis.

REFERENCES

Akem, C.N., 2006. Mango anthracnose disease: Present status and future research priorities. Plant Pathol. J., 5: 266-273.

Al-Rokibah, A.A., M.Y. Abdalla and Y.M. El-Fakharani, 1998. Effect of water salinity on *Thielaviopsis paradoxa* and growth of date palm seedlings. J. King Saud Univ. Agric. Sci., 10: 55-63.

Baird, R.E., 2004. Laboratory Exercises Illustrating Some Fungi in Deuteromycota. In: Plant Pathology, Concepts and Laboratory Exercises, Trigiano, R.N., M.T. Windham and A.S. Windham (Eds.). CRC Press, New York, pp: 141-149.

Barba, J.T., E.M. Reis and C.A. Forcelini, 2002. Effect of temperature and fungicide on the transmission of *Bipolaris sorokiniana* from seeds to barley plants. Fitopatol. Brasileira, 27: 500-507.

Bischoff, T.W. and M.O. Garraway, 1987. Effects of glycose on NADP and NAD glutamate dehydrogenase activities and their relation to ammonium and pH levels in cultures of *Bipolaris maydis* race T. Mycopathologia, 98: 141-148.

Bosch, J.R., A.R. Gonzaleza, J.K. Mitchell and J.M. Aselage, 1992. Quality changes in peach puree by brown rot (*Monilinia fructicola*) and biocontrol agents. J. Food Qual., 15: 449-458.

Crane, J.H. and C.F. Balerdi, 2005. Pitaya growing in the Florida home landscape. IFAS Extension, HS1068, pp: 1-9. http://edis.ifas.ufl.edu/pdffiles/HS/HS30300.pdf.

Davis, R.D., J.A.G. Irwin, D.F. Cameron and R.K. Shepherd, 1987. Epidemiological studies on the anthracnose diseases of *Stylosanthes* spp. caused by *Colletotrichum gloeosporioides* in North Queensland and pathogenic specialization within the natural fungal populations. Aust. J. Agric. Res., 38: 1019-1032.

Dikin, A., K. Sijam, J. Kadir and I.A. Seman, 2007. Mode of action of antimicrobial substances from *Burkholderia multivorans* and *Microbacterium testaceum* against *Schizophyllum commune* Fr. Int. J. Agric. Biol., 9: 311-314.

Elmer, W.H., 2003. Local and systemic effects of NaCl on root composition, Rhizobacteria and *Fusarium crown* and root rot of Asparagus. Phyopathology, 93: 186-192.

Estrada, A.B., J.C. Dodd and P. Jeffries, 2000. Effect of humidity and temperature on conidial germination and appressorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides*. Plant Pathol., 49: 608-618.

FAO, 2004. Fruit of Vietnam. Regional Office for Asia and the Pacific. Food and Agriculture Organization, Bangkok, Thailand.

Fayzalla, E.S.A., Y.M. Shabana and N.S. Mahmoud, 2008. Effect of environmental conditions on wilting and root rot fungi pathogenic to solanaceous plants. Plant Pathol. J., 7: 27-33.

Fitzell, R.D. and C.M. Peak, 1984. The epidemiology of anthracnose disease of mango: Inoculum sources, spore production and dispersal. Ann. Applied Biol., 104: 53-59.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn., John Wiley and Sons Inc., New York, ISBN-10: 0471870927.

Halimi, M.A. and M.G.M. Satar, 2007. Mata Naga. Dewan Bahasa dan Pustaka Publishers, Kuala Lumpur, pp: 74. ISBN: 978-983-62-9795-2.

Hodges, C.F., 1975. Comparative total and proportional rate of germination of *Bipolaris sorokiniana* and *Curvularia geniculata* conidia is influenced by culture age and temperature. Mycopathologia, 57: 9-14.

Hopkins, K.E. and M.P. McQuilken, 2000. Characteristics of *Pestalotiopsis* associated with hardy ornamental plants in the UK. Eur. J. Plant Pathol., 106: 77-85.

Irwin, J.A.G., D.F. Cameron and D. Ratcliff, 1984. Influence of environmental factors on the development of the anthracnose disease of *Stylosanthes* sp. Aust. J. Agric. Res., 35: 473-478.

Janisiewicz, W.J. and J. Roitman, 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phyopathology, 78: 1697-1700.

Jayaswal, R.K., M. Fernandez, R.S. Upadhyay, L. Visintin and M. Kurz *et al*., 1993. Antagonism of *Pseudomonas cepacia* against phytopathogenic fungi. Curr. Microbiol., 26: 17-22.

Kadir, J., M.A. Rahman, T.M.M. Mahmud, R.A. Rahman and M.M. Begum, 2008. Extraction of antifungal substances from *Burkholderia cepacia* with antibiotic activity against *Colletotrichum gloeosporioides* on Papaya (*Carica papaya*). Int. J. Agric. Biol., 10: 15-20.

Le Bellec, F., F. Vaillant and E. Imbert, 2006. Pitahaya (*Hylocereus* spp.): A new fruit crop, a market with a future. Fruits, 61: 237-250.

MacDonald, J.D., 1982. Effect of salinity stress on the development of *Phytophthora* root rot of Chrysanthemum. Phyopathology, 72: 214-219.

Maccheroni, W. Jr., W.L. Araújo and J.L. Azevedo, 2004. Ambient pH-regulated enzyme secretion in endophytic and pathogenic isolates of the fungal genus *Colletotrichum*. Sci. Agric., 61: 298-302.

Masyahit, M., K. Sijam, Y. Awang and M.G.M. Satar, 2008. Fungi Associated with Diseases on Dragon Fruit (*Hylocereus* spp.) in Peninsular Malaysia. Pekan Pahang Publishers, Malaysia, pp: 234-237.

Masyahit, M., K. Sijam, Y. Awang and M.G.M. Satar, 2009. The first report of the occurrence of anthracnose disease caused by *Colletotrichum gloeosporioides* on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia. Am. J. Applied Sci., 6: 902-912.

Palmateer, A.J., R.C. Ploetz, E. van Santen and J.C. Correll, 2007. First report of anthracnose caused by *Colletotrichum gloeosporioides* on Pitaya. Plant Dis., 91: 631-631.

Paull, R.E., 2007. Dragon Fruit. Department of Tropical Plant and Soil Sciences University of Hawaii, Manoa, Honolulu.

Percich, J.A., R.F. Nyvall, D.K. Malvick and C.L. Kohls, 1997. Interaction of temperature and moisture on infection of wild rice by *Bipolaris oryzae* in the growth chamber. Plant Dis., 81: 1193-1195.

Phillips, D.J., 1984. Effect of temperature on *Monilinia fructicola* conidia produced on fresh stone fruits. Plant Dis., 68: 610-612.

Rahman, M.A., J. Kadir, T.M.M. Mahmud, R. Abdul Rahman and M.M. Begum, 2007. Screening of antagonistic bacteria for biocontrol activities on *Colletotrichum gloeosporioides* in papaya. Asian J. Plant Sci., 6: 12-20.

Sijam, K. and A. Dikin, 2005. Biochemical and physiological characterization of *Burkholderia cepacia* as biological control agent. Int. J. Agric. Biol., 7: 385-388.

Sijam, K., Y. Awang and M.G.M. Satar, 2008. Fungi associated with diseases on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia. Proceedings of the Microbes: Biotechnology Engine for Health and Wealth Creation. Hyatt Regency Resort, Kuantan, Pahang Darul Makmur, Malaysia, Aug. 16-19, Malaysian Society for Microbiology, pp: 234-237.

Smith, D.H. and C.L. Fergus, 1971. Effect of light intensity on sporulation of *Botryosphaeria ribis*. Mycopathol. Mycol. Appl. 45: 311-315.

Taba, S., D. Mikami, K. Takaesu, A. Ooshiro and Z.I. Moromizato *et al*., 2006. Anthracnose of pitaya (*Hylocereus undatus*) by *Colletotrichum gloeosporioides*. Jap. J. Phytopathol., 72: 25-27.

Taba, S., N. Miyahara, K. Nasu, T. Takushi and Z.I. Moromizato, 2007. Fruit rot of strawberry pear (pitaya) caused by *Bipolaris cactivora*. J. General Plant Pathol., 73: 374-376.

Tandon, R.N. and S. Chandra, 1962. The nutrition of *Colletotrichum gloeosporioides* Penz. Mycopathologia, 18: 213-224.

Valencia-Botin, A.J., J.S. Sandoval-Islas, E. Cardenas-Soriano, T.J. Michallides and G. Rendon-Sanchez, 2003. *Botryosphaeria dothidea* causing stem spots on *Hylocereus undatus* in Mexico. Plant Pathol., 52: 803-803.

Wang, C.L. and C.C. Lin, 2005. Fruit rot of pitaya dan stem rot of cacti in Taiwan. Plant Pathol. Bull., 14: 269-274.

Wastie, R.L., 1972. Secondary leaf fall of *Hevea brasiliensis*; meteorological and other factors affecting infection by *Colletotrichum gloeosporioides*. Ann. Applied Biol., 72: 283-293.

Xu, X.M., L. Guerin and J.D. Robinson, 2001. Effects of temperature and relative humidity on conidial germination and viability, colonization and sporulation of *Monilinia fructigena*. Plant Pathol., 50: 561-568.