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IMPROVEMENT OF SOILLESS MEDIA BY INCORPORATION OF TRICHODERMA INOCULANT FOR THE PRODUCTION OF CAULIFLOWER

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IMPROVEMENT OF SOILLESS MEDIA BY INCORPORATION OF *TRICHODERMA* INOCULANT FOR THE PRODUCTION OF CAULIFLOWER

By

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Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Agricultural Science in the Faculty of Agriculture
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DEDICATED TO:

MY PARENTS,

SISTERS,

BROTHERS,

AND FRIENDS.

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Agricultural Science.

IMPROVEMENT OF SOILLESS MEDIA BY INCORPORATION OF *TRICHODERMA* INOCULANT FOR THE PRODUCTION OF CAULIFLOWER

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Chairperson : Professor Dr. Sariah Meon

Faculty : Agriculture

Improvement of soilless media by incorporation of *Trichoderma* (UPM 23) for the production of cauliflower under Protected Environment Agriculture (PEA) system was attempted. Three soilless media; coconut dust (CD), palm oil empty fruit bunch (EFB) and peat (P) singly and as mixtures; coconut dust + peat (CDP) and palm oil empty fruit bunch + peat (EFBP) were used in the study. The physical, chemical and microbiological properties of these soilless media showed that their properties were suitable for growth of cauliflower and proliferation and survival of *Trichoderma*. They have good pore size with bulk density within the range of mineral soil (1.0 – 1.8 g/l). CD and CDP have high water availability of 15.18% and 13.99% respectively. However, they have low microbial populations comprising mainly of the genera *Penicillium*, *Aspergillus* and *Rhizopus*. No beneficial microbes were detected

The effect of soilless media on survival and proliferation of *Trichoderma* was carried out by either adding the inoculant as an additive to the soilless media or as bio-seed treatment. *Trichoderma* population in EFB, EFBP and P was reduced more than 50% after 3 days of application. However, in CD and CDP the reduction was only 16.7 % and 34.7% respectively throughout the 21 days of experimental period. *Trichoderma* population was higher on the roots after day 10 of sampling, suggesting that the antagonist can colonize the germinating roots and live on the root exudates. Incorporation to soilless media was a better delivery system for the antagonist as it gave better distribution and easy contact with the growing roots. Therefore, CD and CDP were selected as substrates to evaluate the effect of air-dried preparation of UPM 23 on growth of cauliflower; applied as additive to germination mixes.

Trichoderma inoculant at the rate of 30 x 10^6 cfu/g dry weight substrate have a significant effect on seed germination and seedling emergence of cauliflower. Seedling emergence was 81.3% and 86 38% in CDTV and CDPTV as compared to 40.36% and 42.63% in CD and CDP respectively. Initial establishment and growth of the seedlings were better in *Trichoderma* amended media, as shown by the stomatal conductance and net photosynthesis rate However, there was no significant difference in vegetative growth, nutrient status and peroxidase activity between treatments, probably due to the dilution effect of *Trichoderma* inoculant in the substrate with time The plants were also supplied with full strength Cooper Solution at rate of 600 ml/day

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains Pertanian.

PEMBAIKAN MEDIA TANPA TANAH DENGAN PENGGABUNGAN INOKULUM *TRICHODERMA* UNTUK PENGHASILAN KOBIS BUNGA

Oleh

ISMAIL IBERAHIM

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Pembaikan media tanpa tanah dengan penggabungan *Trichoderma* (UPM23) untuk penghasilan kobis bunga di bawah Pertanian Persekitaran Terkawal telah dijalankan. Tiga jenis media tanpa tanah; sabut kelapa (CD), tandan kosong kelapa sawit (EFB) dan peat (P) digunakan berasingan atau sebagai campuran; sabut kelapa + peat (CDP) dan tandan kosong kelapa sawit + peat (EFBP) digunakan dalam kajian ini. Sifat-sifat fizik, kimia dan mikrobiologi media tanpa tanah menunjukkan bahawa ia sesuai untuk pertumbuhan kobis bunga serta pembiakan dan keupayaan hidup *Trichoderma*. Ia mempunyai saiz rongga yang baik dengan ketumpatan pukal berada dalam lingkungan tanah mineral (1.0-1.8 g/l). CD dan CDP mempunyai peratus kandungan air tersedia yang tinggi iaitu 15.18% dan 13.99%. Walaubagaimana pun ia mempunyai populasi mikrob yang rendah terutamanya terdiri dari genera *Penicillium*, *Aspergillus* dan *Rhizopus*. Tiada mikrob-mikrob berguna dapat dikesan.

Kesan media tanpa tanah ke atas pembiakan dan keupayaan hidup *Trichoderma* telah dijalankan samada sebagai tambahan kepada media tanpa tanah atau rawatan biji benih. Populasi *Trichoderma* dalam EFB, EFBP dan P telah menurun lebih dari 50%, tiga hari selepas aplikasi. Walaubagaimana pun dalam CD dan CDP peratus penurunan hanya sebanyak 16.7% dan 34.7% masing-masing sepanjang 21 hari tempoh ujikaji. Populasi *Trichoderma* adalah tinggi pada akar selepas 10 hari persampelan, menunjukkan bahawa antagonis tersebut boleh mengkolonisasi akar yang bercambah dan boleh hidup pada cecair rembesan akar. Penambahan inokulum kepada media tanpa tanah memberi taburan lebih baik dan mudah untuk bersentuh dengan akar yang tumbuh. Oleh itu CD dan CDP telah dipilih sebagai substrat untuk menilai kesan persediaan UPM 23 secara kering udara ke atas pertumbuhan kobis bunga; digunakan sebagai tambahan kepada campuran cambahan.

Trichoderma pada kadar 30 x 10^6 cfu/g berat kering substrat mempunyai kesan yang beerti terhadap percambahan biji benih dan kemunculan anak benih. Peratus percambahan anak benih adalah 81.3% dan 86.38% dalam CDTV dan CDPTV dibandingkan dengan kawalan sebanyak 40.36% dan 42.63% masing-masing dalam CD dan CDP. Pertumbuhan peringkat awal anak benih lebih baik dalam media yang telah digabungkan dengan *Trichoderma*, sebagaimana ditunjukkan oleh koduksi stomatal dan kadar fotosintesis bersih. Walaubagaimana pun tiada perbezaan beerti pada pertumbuhan vegetatif, status nutrien dan aktiviti peroxidase di antara rawatan yang mungkin disebabkan oleh kesan pencairan inokulum *Trichoderma* di dalam substrat dengan masa. Pokok juga diberi baja Cooper Solution yang mencukupi iaitu pada kadar 600 ml/hari.

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CHAPTER 1

INTRODUCTION

As Malaysia becomes more affluent and industrialised, the per capita consumption of vegetables will automatically increase. The current per capita consumption of vegetables in Malaysia is only 36 kg compared to 70 kg and 136 kg respectively for Singapore and Japan (Leong *et al.*, 1994). There is a great potential for higher vegetable consumption in the future. However, the production of vegetables, particularly high value crops such as cabbage and cauliflower has shown marginal increase.

In 1995, total area planted with cauliflower was only 80 ha with total production of about 480t. Import of this vegetable alone was estimated as 13,598t which amounted to RM 60 millions (Kementerian Pertanian Malaysia, 1996). Hence, the government has embarked on an aggressive campaign to increase the production locally.

Cauliflower (*Brassica oleracea* var *botrytis*) is one of the popular cruciferous vegetables in Malaysia and commonly confined to the highlands. The rapid development towards industralization, housing, and other activities such as golf course and recreation has put pressure on the availability of agricultural land. Due to this reason, cauliflower is grown in the lowlands, near to highly populated areas.

Research has been carried out to produce this vegetable crop in the lowlands with the adoption of the protected environment production system using netted and rain shelters. An important factor for the success of this system is the selection of the varieties for production. Heat resistant and short maturity period had been identified as important criteria in selecting suitable varieties for planting. Maturity period of less than 70 days for cauliflower was ideal for this purposes (Syed Abdul Rahman *et al.*, 1987; Fadelah *et al.*, 1990). The varieties Hybrid F1 451, Chia Tai, Snow Top and Summer Crown were recommended for planting under rain shelters in the lowland. Research conducted by MARDI also recorded higher yield when cauliflowers were planted in soil with high peat content.

The major pest associated with cauliflower is diamondback moth (*Plutella xylostella*) which also attack other cruciferous crops. The unlimited and indiscriminate use of chemicals cause high level of resistance detected to melathion, DDT, chlorpyrifos-methyl, lindane and dichlorvos (Syed, 1990). Diseases of cauliflower are mainly caused by soil-borne pathogens such as clubroot (*Plasmodiophora brassicae*), basal rot (*Rhizoctonia solani*) and seedling damping-off (*Pythium* and *Phytophthora* spp) (Dixon, 1984; Ahmad, 1994). The diseases are prevalent in areas of high humidity and dense planting especially during the rainy season. Drip irrigation system can reduce disease incidences. However, the non-availability of good topsoils have promoted utilization of non-soil materials as media for production in Protected Environmental Agriculture (PEA).

The problem can be overcome by planting cauliflower under netted structures or rain shelters using soilless media, which could also promote the utilization of agro-industrial product such as coconut coir, palm oil mill effluent (POME) or peat for the cultivation of high value crops (Yahya and Razi, 1997). Fertilization is well regulated by the drip irrigation to ensure uniform growth of plants. This system also enables the production of vegetables in a short period of time compared to the conventional method of soil cultivation. The vegetables are high in quality with minimum or none pesticide residue, which is considered to be harmful to the environment and consumers. In addition, consumers generally want cheap food produced by a system that used less chemicals.

The movement from soil-based growing system to soilless culture such as sawdust and peat and the recirculating nutrient film technique meant that plants grown under these soilless media will have lower population of microorganisms in the rhizosphere, including those beneficial for plant growth or disease suppression. Opportunity exists, then, to introduce beneficial microbes into this soilless media.

Research has been carried out with biological preparations (products) in soil and artificial substrates to stimulate plant growth such as the use of commercial preparations of effective microorganisms. *Trichoderma* spp have been shown to have a growth stimulating effect as well as good bioprotectants against many soil-borne diseases (Baker, 1989; Lewis and Larkin, 1997). Previous studies have shown that *Trichoderma* enhance seed germination, root and vegetative growth and flower production (Chet, 1987). It also enhanced rooting of cuttings, promote growth as measured by increase in weight, height and branch, and flower production as compared with controls for chrysanthemums and petunias (Chang *et al.*, 1986; Paulitz, 1997). Cellulose granules containing isolates of *Trichoderma* and

Gliocladium reduced root diseases of eggplants by *Rhizoctonia solani* and resulted in higher stands comparable to that in the non-infested soilless mix (Lewis *et al.*, 1998).

In view of this, the present study was undertaken with the aim of improving the organic-based media for the production of cauliflower by incorporation of air-dried preparation of *Trichoderma* inoculant.

CHAPTER 2

LITERATURE REVIEW

Cauliflower Crops

Cauliflower (*Brassica oleracea* var *botrytis*) belongs to the family of Cruciferae. This crop was known to originate from the Mediterranean and growing well in the temperate region (Syed Abdul Rahman *et al.*, 1987). Cauliflower was first planted in Cameron Highlands in the 1950's. However, in 1995, the total area planted has increased to 80 ha with total production of about 480 t (Kementerian Pertanian Malaysia, 1996). Maximum temperature for curd initiation is in range of 20 - 25°C and the optimum temperature is 18°C. Soil with high content of peat, well drained and pH 6.0 - 6.5 are good for planting cauliflower (Syed, 1990).

Among the Brassica crops, cauliflower has the most exacting climatic and cultural requirement. Cauliflower is much more intolerant to frost and high temperature. Cultivars known as "tropical types" having better warm-temperature adaptation are grown in large volume in many warmer temperate regions, namely India and China (Hartmann *et al.*, 1988). The edible product called the head or curd is mistakenly considered to be floral tissue, but is instead prefloral fleshy apical meristem tissues. The curd consist of the repeatedly branched terminal portion of the main axis of the plant, comprised of a shoot system with short internodes, branch apices and bracts. High temperature result in poor head formation and quality with accompanying market defects such as bract development within the curd, loss of compactness and ricy curds. Direct exposure of the curds to sunlight tends to discolour the otherwise white curd surfaces to a creamy yellow, which is a market defect but does not otherwise affect its edible quality. To protect and blanch the enlarging curd, the long outer leaves are normally gathered together over the top of the curd and tied together to keep out the light (Hartmann *et al.*, 1988; http://www.orst.edu/Dept/NWREC/cauli.html, 2000).

The low production and quality of cruciferous vegetables in Malaysia are due to the hot and wet climate, pest and diseases, and inefficient agronomic management (Illias and Ramli, 1994). Cruciferous grown in the Cameron Highlands are often damage in the field by a number of diseases such as basal rot (*Rhizoctonia solani*), *Altenaria* leaf spot (*Altenaria brassicae*), black rot (*Xantomonas campestris*) and clubroot (*Plasmodiophora brassicae*). Of the four diseases mentioned, clubroot is the most important disease of cruciferous in the Cameron Highlands (Ahmad, 1994). The major pest of crucifers is *Plutella xylostella*. Loss from the damage caused by *Plutella* was very high and up to 100% has been reported (Fauziah *et al.*, 1997). Minor pests include *Hellula undalis*, *Spodoptera litura*, *Crocidolomia binotalis*, *Agrotis ypsilon*, *Phyllotreta* spp and *Lipephis erysimi*.

The variation in pH and temperature, and sometimes nutrient factors can cause physiological disorder Buttoning is common in curd before maturation This is due to lack of nitrogen, dense planting or delay in transplanting. Water-soaked lesions on the curd and stem called browning are usually associated with boron deficiency. Bracts or small leaf are formed on the curd because of high temperature during and after curd initiation (Syed Abdul Rahman *et al.*, 1987).

Trichoderma **spp**

Trichoderma spp belongs to the Mitosporic fungi (Hawksworth *et al.*, 1995). The colony grew well on Potato Dextrose Agar (PDA) with smooth, white and clear surface at first, later turning into greenish colour. It is classified as an imperfect fungi that produced one-celled conidia or chlamydospore on branched conidiophore. The phialides were produced at the end of the conidiophore with phialospore at the end of each phialide (Rifai, 1969; Koh, 1990). Most *Trichoderma* spp are not encountered in association with sexual stages and are considered to be strictly mitotic clonal fungi.

As expected with saprophytic soil fungi, *Trichoderma* use a wide range of compounds as sole carbon and nitrogen sources. The carbon and energy requirements of *Trichoderma* can be satisfied by monosaccharides and disaccarides; complex polysaccharides, purines, pyrimidines and amino acids, particularly long-chain fatty acids and even methanol, methylamine and formate (Papavizas, 1985).

Most species of *Trichoderma* are photosensitive, sporulating readily on many natural and artificial substrates in a concentric pattern of alternating rings in response to diurnal alternation of light and darkness, with conidia being produced