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Research investigation on microbial technology for plant disease control: a short communication

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Abstract *Chaetomium* spp. are isolated, identified and screened to control plant pathogen isolated in China. Disease interacting pathogens were found in peach diseases which were *Pythium, Rhizoctonia solani* (twig die back), *Fusarium* spp (wilt) *Phomopsis*, *Colletotrichum dematium*, *Colletotrichum gleosporiodes*, *Phoma*, *Fusarium, Fusarium*, *Phomopsis*, and *Alternaria*. The main primary organism caused infection to peach supposing to be *Phomopsis* and *Fusarium* destroying roots and vascular bundle, and the pathogens infected above peach tree in stem, twigs and leaves found *Colletotrichum dematium*, *Colletotrichum gleosporiodes*, and *Alternaria*. Bi-culture test between *Chaetomium* antagonistic fungus and phytopathogens was evaluated to know the control mechanism which tended to give good results. Futher testing bioproduct of *Chaetomium* had been also tested in tea, peach, grape and old trees with different phytopathogenic isolates and reveiced a good result to control and recovery plants from disease incidence.

Keywords: Biocontrol, Plant diseases, Phytopathogens

Introduction

There are reported that the potent microorganism isolated in China is expressed to promote the plant growth and showed high degree of antagonistic activity against plant pathogens eg *Pythium, Fusarium, Colletotrichum*, *Phomopsis, Alternaria* etc. *Bacillis subtilis* is also isolated from rhizosphere soil and found to be antangonize phytopsthogens. Both *Chaetomium* sp**.** and *Bacillus* sp are further developed to be biopreparates used in peach, citrus, grape, tea and other oplants (Song and Soytong, 2018). *Chaetomium* species belongs to *Chaetomiaceae*, Ascomycota (Kunze and Schmidt (1817). It is a largest species of saprophytic ascomycetes over 300 species worldwide (von Arx *et al*., 1986). *Chaetomium* is antagonized the growth of bacteria and fungi

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(Zhang and Yang, 2007). *Chaetomium globosum* and *Chaetromium cochlioides* inhibited *Fusarium* spp. and *Helminthosporium* spp. (Tveit and Moore, 1954), *Pythium ultimum* (damping-off of sugar beet) (Di-Pietro *et al*., 1991), *Rhizoctonia solani* (Walter and Gindrat, 1988), leafblight of brassicas (*Alternaria brassicicola*) (Vannacci and Harman, 1987) and *Botrytis cinerea* (deadly lily leaves) (Kohl *et al.*, 1995). *Chaetomium cupreum* controlled soybean pathogens e.g. *Phomopsis* and *Colletotrichum* spp. (Manandhar *et al.*, 1986). *Ch. cupreum* and *Ch. globosum* controlled leaf spot of corn (*Curvularia lunata*), rice blast (*Pyricularia oryzae*), rice sheath blight (*Rhizoctonia oryzae*) (Soytong, 1992). *Ch. globosum* controlled the apple scab *Venturia inequalis* causing apple scab (Cullen *et al*., 1984). *Ch. cupreum* inhibited *Phomopsis sojae* in soybean (Manandhar *et al.*, 1986). *Ch. globosum* inhibited *Fusarium oxysporum* f. sp. *lycopersici* and *Pseudomonas solanacearum* and controlled *Thielaviopsis* Bud Rot of Bottle palm caused by *Hyophorbe lagenicaulis* (Soytong *et al.*, 2005). *Ch. globosum* and *Ch. cupreum* are successfully applied to control root rot disease of citrus, black pepper, strawberry and damping off disease of sugar beet (Soytong *et al.*, 2001; Tomilova and Shternshis, 2006). It was recorded that to control raspberry spur blight (*Didymella applanate*) and reduced potato disease (*Rhizoctonia solani*) and increasing potato yield (Shternshis *et al*., 2005). pathogens in higher doses (Tomilova and Shternshis, 2006). It is noted that Chaetomium biofungicide has been proved to control several diseases in the fields to control black paper (Sodsa-art and Soytong, 1999), root rot of durian (Prechaprome and Soytong, 1997). *Ch. globosum* reduced the inoculum of *Diaporthe phaseolorum* f. sp. *meridionalis* in soil-surface soybean stubble in field conditions (Dhingra *et al*., 2003). *Ch. globosum* expressed a role of antibiosis to control of spot blotch (*Cochliobolus sativus*) of wheat (Aggarwal *et al.,* 2004). Chaetomium spp produce cellulase to degrade cellulose, lignin and organic materials (Soytong *et al*., 2001). Several bioactive pure compounds are found from *Chaetomium* spp e.g. benzoquinone derivatives (Brewer *et al*., 1968). *Chaetomium udagawae* recorded to produce a new producer of sterigmatocystin. Udagawa *et al*, 1979). *Ch. globosum* produces chaetoviridins which antifungal activity against plant pathogenic fungi. Chetomanone, chaetoglobosin C, echinuline and isochaetoglobosin D were produced from *Ch. globosum* KMITL-N0802 and chaetomanone and echinulin expressed bioactivity towards *Mycobacterium tuberculosis* (Kanokmedhakul *et al*., 2002). The research findings were to isolate the potent microorganism especilaaly Chaetomium and to screen plant disease control in China.

Research development in Guo Fengyanong in Nantong

Isolation of Chaetomium antagonistic fungus and phytopthogens

Chaetomium antagonistic fungus is isolated by baioting techniques from cultivated soil samples and using the sterilized filter papers as baits. The disease samples of peach tree eg, twig dieback, gummosis, leaf light, rhizosphere soil and root rot were taken from the field in Wuxi, Jiangsu province, and brought to Research Laboratory in Gao Fengyanong Company. Tissue transplanting method was conducted using sterilized advanced margin between diseased and healthly tissues, placed to potato dextrose agar (PDA) plates, incubated at room temperature. Hyphal tip isolation was done until get pure culture. Pure culture was moephologically identified. Baiting technique was conducted by using pieces of healthy tissue piececs placed to the palates containing soil sample and sterilized water, incubated at room temperature for 1-2 days. Mycelia and fungal structures were observed under compound microscope to get pure cultures of either Phytophthora or *Pythium* spp.

Bi-culture test

The agar plugs of *Chaetomium* antagonistic fungi and the pathogen were transferred to PDA plates at equal distance of each side. Control plates were separately cultured either Chaetomium antagonistic fungus or pathogen in PDA plates. Experiment was arranged in Completely Randomized Design (CRD) with four replications.

Testing *Chaetomium* to control the disease complex of Peach in Wuxi

Chaetomium cuporeum CC, *Chaetomium globosum* Cg and *Chaetomium cochlioildes* CH were isolated and morphologically identified. It is found that disease complex of peach caused by many pathogens. Isolation of pathogens were found *Pythium, Rhizoctonia solani, Colletotrichum gloeosporiodes, Colletotrichum dematium, Fusarium oxysporum, Phomopsis* spp., *Alternaria*, and *Phoma* etc. Bi-culture tests were investigated to screen different isolates of Chaetomium spp which were *Ch. globosum*, *Ch. cupreum* and *Ch. cochliodes*. Three species of *Chaetomium* were tested against phytopathogens isolated from peach diseases called disease interaction or disease complex as seen in Figures 1 and 2. Result revealed that *Ch. globosum* CN1 gave a good result to inhibit *F. solani* 1 (51.53 %), and followed by *C. dematium* (39.72%), *C. gloeosporiodes* 1(36.94%), *C. gleosporiodes* 2 (36.81%), *F. oxysporum* (35.42), *Phoma* (35.46%), Phomopsis (35.97%) and *Alternaria* (37.22%) as seen in Figure 1 and Table 1). It showed that *Chaetomium cupreum* CN 2 gave a good inhibition of *C. dematium* (38.61%), and followed by *F.solani* 1 (33.61*), F.oxysporum*

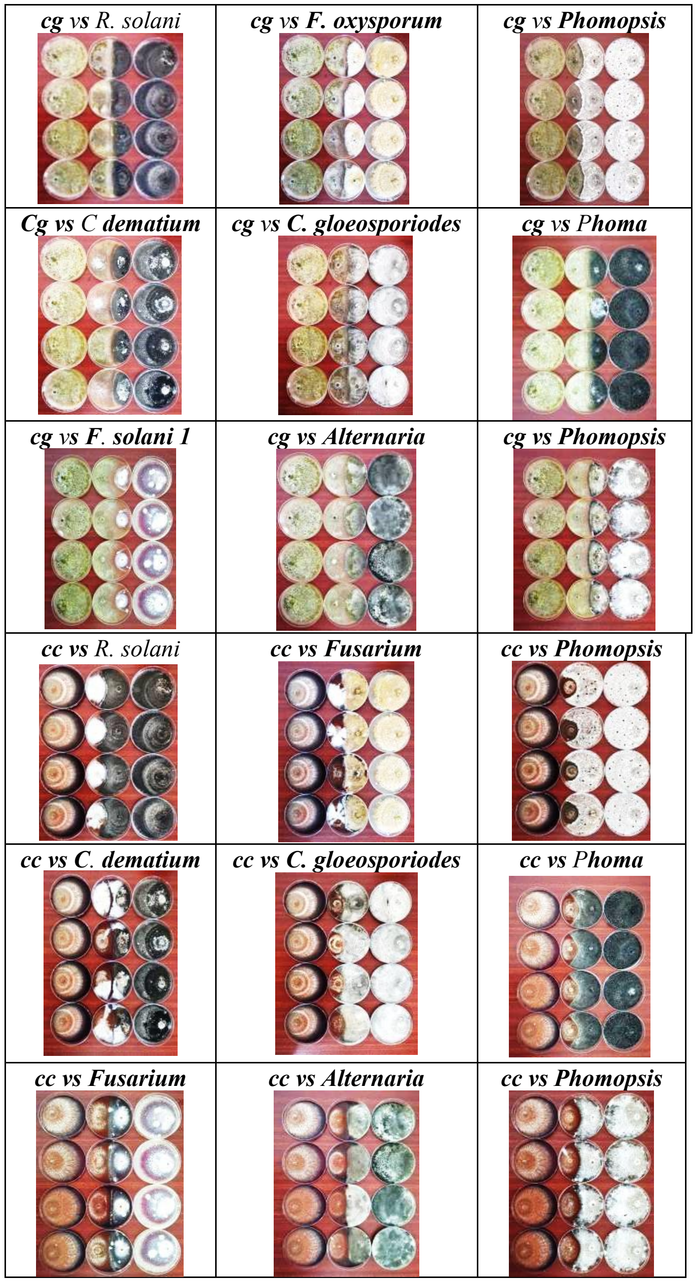

Figure 1. *Chaetomium globosum* Cg and *Chaetomium cupreum* CC testing against phytopathogens from peach

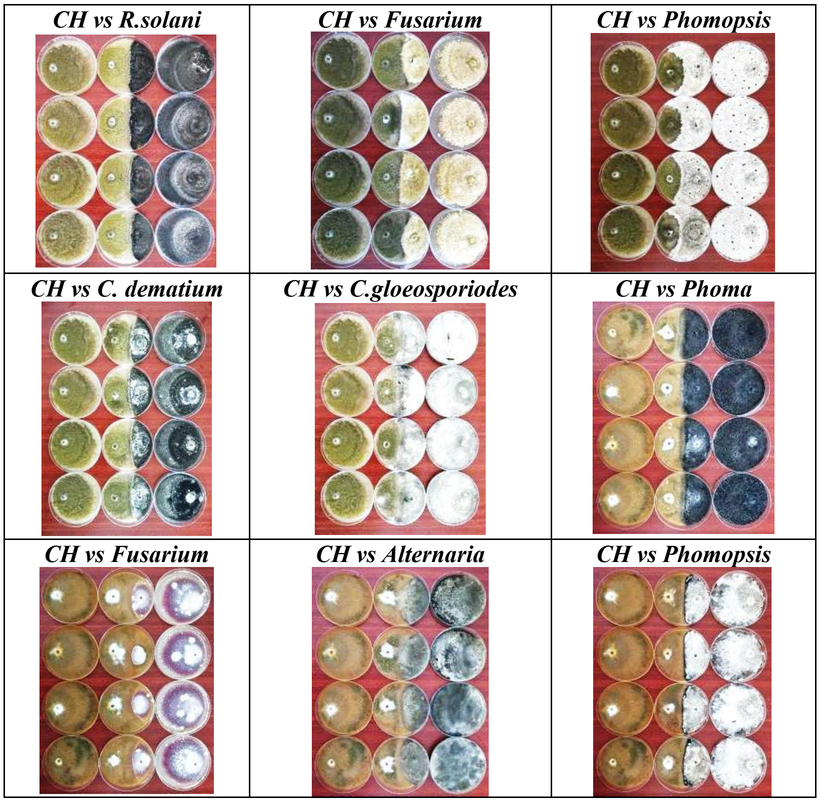

Figure 2. *Chaetomium cochliodes* CH testing against phytopathogens from peach

Table 1. *Chaetomium globosum* Cg testing against phytopathogens from peach in bi-culture investigation for 30 days

Treatments	R1	R2	R3	R4	X	mean	%inhibit
Control	9.00	9.00	9.00	9.00	9.00	9.0^{a1}	-
C. glooeosporiodes 1	5.70	5.65	5.6	5.75	5.68	5.67^{d}	36.94^{c}
F.oxysporum	5.85	5.85	5.8	5.75	5.81	5.81^{b}	35.42^{c}
Phomopsis	6.25	6.25	6.1	6.25	6.21	6.21^{d}	30.97^{e}
C. dematium	5.45	5.5	5.4	5.35	5.43	5.43^{e}	39.72^{b}
C. gleosporiodes 2	5.75	5.65	5.75	5.60	5.69	5.69^{d}	36.81^{c}
Phoma	5.85	5.75	5.75	5.85	5.80	5.80^{d}	35.56^{c}
F.solani 1	4.50	4.00	4.55	4.40	4.36	4.36^{f}	51.53^{a}
F. solani 2	6.05	6.05	6.00	5.95	6.01	6.01^{c}	32.19^{d}
Phomopsis,	5.70	5.65	5.85	5.85	5.76	5.76^{d}	35.97^{c}
Alternaria	5.70	5.65	5.60	5.75	5.65	5.65^{d}	37.22^{c}
C.V.	-	-	-	-	-	1.69	3.15

[1]Averaged data from fosur repeated experiment and followed by a common letter are siggnificantly differed by DMRT at P=0.01.

Table 2. *Chaetomium cupreum* CC testing against phytopathogens from peach in bi-culture investigation

Treatments	R1	R2	R3	R4	X	mean	%inhibit
Control	9.00	9.00	9.00	9.00	9.00	9.00^{a1}	-
C. glooeosporiodes 1	6.15	6.05	6.25	6.15	6.15	6.15^{e}	31.67^{c}
F.oxysporum	6.00	5.95	6.00	6.15	6.03	6.03^{f}	33.06^{b}
Phomopsis	6.30	6.30	6.20	6.30	6.28	6.28^{d}	30.28^{d}
C. dematium	5.45	5.55	5.60	5.50	5.53	5.53^{g}	38.61^{a}
C. gleosporiodes 2	6.35	6.30	6.40	6.45	6.38	6.38^{c}	29.17^{e}
Phoma	6.35	6.40	6.45	6.45	6.41	6.41^{bc}	28.75^{ef}
F.solani 1	6.05	6.00	5.95	5.90	5.98	5.98^{f}	33.61^{b}
F. solani 2	6.50	6.04	6.55	6.55	6.50	6.50^{b}	27.78^{f}
Phomopsis	6.15	6.30	6.15	6.20	6.20	6.20^{de}	31.11^{cd}
Alternaria	6.25	6.15	6.10	6.15	6.16	6.16^{e}	31.52^{c}
C.V.						1.00	2.37

[1]Averaged data from fosur repeated experiment and followed by a common letter are siggnificantly differed by DMRT at P=0.01.

Table 3. *Chaetomium cochliodes* CH testing against phytopathogens from peach in bi-culture investigation

Treatments	R1	R2	R3	R4	X	mean	%inhibit
Control	9.00	9.00	9.00	9.00	9.00	9.00^{a1}	-
C. glooeosporiodes 1	5.40	5.50	5.95	5.10	5.35	5.35^{f}	40.56^{b}
F.oxysporum	5.70	5.90	6.35	5.60	5.79	5.79^{de}	35.69^{c}
Phomopsis	6.15	6.30	5.25	6.15	6.24	6.24^{b}	30.69^{e}
C. dematium	5.45	5.25	6.10	5.50	5.36	5.36^{f}	40.42^{b}
C. gleosporiodes 2	6.25	6.10	5.95	6.20	6.16	6.16^{bc}	31.53^{de}
Phoma	6.10	6.00	4.30	5.90	5.99	5.99^{bc}	33.47^{cd}
F.solani 1	4.50	4.90	6.10	4.00	4.43	4.43^{g}	50.83^{a}
F. solani 2	6.00	6.00	5.75	6.10	6.05	6.05^{bc}	32.78^{de}
Phomopsis,	5.70	5.80	5.75	5.75	5.75	5.75^{e}	36.11^{c}
Alternaria	5.75	5.80	5.95	5.75	5.76	5.76^{de}	35.97^{c}
C.V.	-	-	-	-	-	2.49	4.73

[1] Averaged data from fosur repeated experiment and followed by a common letter are siggnificantly differed by DMRT at P=0.01.

Chaetomium biofungicide

All three potent isolates of *Chaetomium* found in China was further synegistized to each other and has beem formulated to be liquid biofunghicide at Guo Fengyanong Company, Nanotong town, P.R. China to be tested in several kinds of plant diseases (data not shown) eg. peach, strawberry and old trees etc. They are evaluating to prove the efficacy for disease control in the fields.

Organic tea demonstration in China

Tea has a long history in China.Some says dringking tea culture started from ancient times, and some saying it has started from Zhou dynasty, the others thinking it has started from Qinhan dyasty. Three kingdoms period, the Northern, Southern, Tang dynasties. It is true that the habits in drinking tea in most other countries are spread from China. In China, there are six series of tea which are green tea, black tea, oolong tea, white tea, yellow tea and dark tea. There are four tea growing regions in China, which are South West Tea growing region, South Tea growing region, South of Yangtze River growing region, and North of Yangtze River growing region. South West Tea growing region is the most ancient growing region in China including Yunnan province, Guizhou Province, Sichuan Province and the Southeast Tibet which mainly produce pu'er tea, Sichuan Mengding yellow tea, Ganlu tea or Duyun Maojian green tea in Guizhou province. South tea growing region includes guangdong, guangxi, southern fujian, southern Yunnan, Hainan which produce Tie Guan Yin tea, Baihaoyinzhen tea, Jasmine tea, Fenghuangdancong tea, and Liubao tea (Figure 3).

Figure 3. Demonstration of organic tea in China

Our experimental tea tested in Damushan Songyang, Zhejiang that is gold bud black tea plantation. We had cooperated with local governments and farmers for biological control of disease and insects in the early 2018 in green tea, white tea and dark tea. We had mainly conducted the experimental organic tea bases in Songyang, Zhejiang (green tea, local wild tea), Yixing (black tea), Anhua. Zhejiang (dark tea) and Anji .zhejiang (white tea and gold bud tea). Our bio-products functions to produce organic tea in Longjing green tea in Songyang, Zhejiang which in early 2019.

Our bioproducts are interval appolied every 10-15 days as bio-agents to control diseases, to increase soil fertility, to improve disease immunity, to stimulate plant growth. The tea had strated to revover after application. Mr liujinfa is one of the tea plantation owner reported to the county government tthat compared to previous tea before application, it can be seen the new healthly leaves after applying biological agents that become more tender and taste much better. Moreover, Huangdu village, Zhejiang is the origin of Anji gold bud tea which had started to apply our bioproducts in the spring of 2018. Thereafter, the sprouting rate of new tea raised more than 20% and the quality also improved a lot compared to last year which used agrochemicals in the early 2019. It got a high yield without toxic chemical pesticides and it would serve the people to drink green with safety life. However, in Yixing Organic Tea is located in Hufu which is closed to Zhanggong Hole Scenic Area which covers 100 mu of yellow tea. There arev lack of good field management, leading to cause seriously anthracnose caused by *Colletotrichum* sp., root wilt disease caused by *Fusarium* sp. and leaf spot disease caused by *Pestalotia* sp. Our bio-productds used to apply to those tea plants in large doses of bio-agents for three times at every 15 days. As a result, the diseases were controlled and the new leaves come out without any dsigns of symptoms. Phong, *et al.* (2016) found that *Chaetomium* spp actively against *Fusarium* wilt of tea in Vietnam.

Recovery the diseases of *Ligustrum lucidum* in Yangzhou

Ligustrum lucidum 's trees are completely recovered with healthy green leaves after application of bio products from 25 December 2018 to 24 April 2019 (about 4 months). Application bio-products are reccommended to adjust the soil pH to 6.5-6.8 by adding lime, sprayed bio-agents (Ketomium, nanoelicitor, and nutrition) to whole plant, under the canopies, and into soil around the root system of plants. The serious disease was strongly applied at highest application rate for three time interval of 3, 5, 7 or 10 days. Apply bio-products around the basal stem and into the made holes into the root system under soil in 19th March 2019, 30th April 2019, 11th March 2019, 19th March 2019 and 19th March 2019, 8th April 2019 and 16th April 2019. Observation after application was found that root and stem stopped rotting, new leaf flush come out. The tree is recovered by the new leaves come out with normal healthly leaves and green (high chlorophyll content), and no twigs die back. The disease problems of *Ligustrum lucidum* are recommended how to solve the problem by biological method (25th December 2018) (Figure 4).

Ligustrum lucidum is an old tree over 100 years appeared root rot symptom. The diseased tree was seriously infected by plant pathogens before application of bioproducts which diagnosed on 11th March 2019. Bio-product of Chaetomium is interval applied at every 7 days until plant recovery. Observation of new twigs and leaves flush come out and growing well on 19th March 2019. The plant canopies are recovered with new twigs and leaves, green and full normal leaves on 8th April 2019. The rotting symptom on basal stems stopped on 24^{th} April 2019 and the new twigs come out more with new leaf flush on 24^{th} April 2019. The completely recovered of tree is seen on 22^{nd} May 2019. Moreoveer, it found some larvae of insect is observed and insect pupa died (19th March 2019) due to bio-insecticide including Metarhizium and Beaveria. It is Cockchafer (*Melolontha melolontha*) destroyed the tree (Figure 5).

Before application	**After application**

Figure 4. Application of *Chaetomium* bioproduct to recobery *Ligustrum lucidum*

Figure 5. Disease recovery of *Ligustrum lucidum*

Citrus disease complex (CDC) in Ganzhou City, Jiangxi province, China

Gannan navel orange is a special variety of Ganzhou City which characterized with big fruits, bright yellow color, sweet taste, good quality and flavor. It is only selected product of Jiangxi province in a shortlist of Ministry of Commerce and listed product in China and EU geographical signed negotiation. Ganzhou City is origin of Gannan navel orange which is the world's first-largest Navel orange plantations and its output ranks to the third in the world. Ganzhou is now the largest navel orange producing area. After disease survey and diagnosis, It found root rot caused by Phytophthora sp., Pythium sp and above plants were nmainly found the symptoms of anthacnose caused by Colletotrichum, canker caused by *Xanthomonas axonopodis* pv *citri,* and nutrient deficiency. Morevover, rhizosphere soil was acidity of pH lower than 5. The diagnosis showed that Citrus disease problem called Citrus disease complex (CDC) meaning many diseases in one plant. The main diseases are root rot casused by *Phytophthora* or *Pythium*, Huabglongbing (HLB) caused by motile bacteria, *Candidatus* Liberibacter .(pholem limited bacteria) which the insect vestor transmitted are psyllid or greening disease. Application of bio-techniques has succesfully recovered the CDC to be healthly citrus trees . The recovered citrus tree from CDC is cleasly seen the new healthly leaves that not appeared any symptoms of HLB or nutrient deficiency. The heathly green leaves and citus trees may still have phoem bacteria without symptom which define as latent infection. As we tested in October 2017, CAS technical team was invited to Pujiang County Sichuan province by Sichuan Best orange company to diagnose and prevent diseases and insect pests for 10 thousand mu of orange plantation . In December 2017, CAS technical team was invited to Ganzhou City Jiangxi Province to prevent Citrus Disease Complex (CDC) of navel oranges. The experimental field was located in Yongquan village, Hubian town in developmental area, Ganzhou city. The third times applied CAS bio-products, it started to see more green leaves. The fourth times applied bio-products, the leaves had more bigger and green. The citrus leaves are recovered over 90 %. The new healthly green leaves and started flowering. The citrus trees are completely recovered from CDC. Until the end of April 2019, result showed that after application of bio-products for 8 times, the citurs trees are recovered from CDC, effectively control, new leaves are healthy and green (Figures 6 and 7). Hung *et al.* (2015) reported that *Chaetomium* sp controll *Phytophthora* sp causing Citrus root rot).

Figure 6. Citurs trees declined before application bioproducts

Figure 7. Citrus tree recovery after applying bioproducts

Grapes in Xinjiang Municipality

The numer five of agricultural division in Xinjiang Municipality has done to build green grape brand in Bole City. The grape trees are applied bio-products including Chaetomium for disease control, Bioinsecticise, and Bio-stimulasts resulted to the grape trees grew out new twigs and buds. Afert repated applications at every 15 days, the grape trees become much better growth than before. The bio-products has good affected on controlling diseases and insect pests with many field trials. During the time by using bio-products, the grapes in Bole City won the gold award in grape competition with beautiful color, tight flesh and sweet taste.

Future perspectives

Biofungicides and biofertilizers has been developed for crop improvement and increase yield (Kaewchai *et al*., 2009). Our research and development on *Chaetomium* biological products have been investigated since 1989. The first *Chaetomium* bioproduct is distributed as a new broad spectrum biological fungicides (Thailand Patent No. 6266, International Code: AO 1N 25/12 which registered as biofungicide to control plant diseases in Thailand, Lao PDR, Vietnam, Cambodia, and being registration in Myanmar and Malaysia etc. *Ch. cupreum* CC01-CC10 and *Ch. globosum* CG01-CG12 in pellets, powder and liquid formulations has developed to be biopreparates. It is successfully applied by farmers to control diseaases in many countries. It is scientifically proved that not only protection but also curative effects and promoting plant growth. The bioproducts can be applied for good agricultural practices (GAP), pesticide free production (PFP), non agrochemical production (NAP) and organic agriculture (OA). *Ch. globosum* YSC5 are reported to be nematicidal metabolite action against plant parasitic nematode (Khan, *et al.*, 2019). Nematicidal Activity of Chaetoglobosin A produced by *Ch. globosum* NK102 reported to against root knot nemastode caused by *Meloidogyne incognita* (Hu *et al.*, 2013). Our further reserch findings are alsop interested to find some other active strain of *Chaetomium* species against plant parasitic nematode. *Chaetoimum* biofungicide, bioinsectide and biostimulants can be applied to implement integrated pest management (IPM). Further research is undergoing on the other bioactive compounds from active strains of *Chaetomium* spp. We have discovered various new compounds from *Ch. globosum*, *Ch. Cupreum*, *Ch. elatum*, *Ch. Cochliodes*, *Ch. brasiliense*, *Ch. lucknowense*, *Ch. longirostre.* and *Ch. siamense*. These species are further developed to be biopreparations for disease control, immunity and plant growth stimulants.

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