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Efficacy test for good agricultural practice, pesticide-free production and organic agriculture in tomato

Sibounnavong, P.S.[1*], Aram Utthajadee[1], Chaiwat Makhonpas[1] and Kasem Soytong[2]

[1]Faculty of Agro-industrial Technology, Rajamangala University of Technology Tawon-ok (RMUTTO), Chanthaburi Campus, Thailand
[2]Biocontrol Research Unit & Mycology Section, Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok, Thailand

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Mycephyt promoted spore production of *Chaetomium cupreum* and *Chaetomium globosum* which act as biological fungicide for disease control. It is undoubledtly stated that Chaetomium-powder can be synergisted to Mycephyt which formulated as CM product. Mycephyt significantly inhibited the conidia production of *F. oxysporum* f sp.*lycopersici*. Comparison of GAP, PFP and organic methods in three varirties of tomato (Cherry, Loukthor and Sida) gave significantly better in growth parameters or plant stands than the non-treated control. It is indicated that organic method with potent bio-products as CM product for disease control, bio-insecticide (Beauveria and Metarhizium), bio-fertilizer would promote plant growth parameters of tomato var Cherry, Loukthor and Sida. The organic method gave significantly highest number of fruits in all tested varieties, followed by PFP and GAP. But in chemical method gave lower number of fruits when compared to organic, PFP, GAP and non-treated one. It is obsereved that the chemnical method and non-treated control seriously faced the wilt incidence caused *F. oxysporum* f sp *lycopersici* thereafter diagnosis and isolated the pathogen to identify, especially Sida variety.

Key words: *Chaetomium cupreum*, *Chaetomium globosum,* Mycephyt, CM product, Cherry, Loukthor and Sida

Introduction

The cultivation of tomato (*Lycopersicon esculatum* Mill.) has been faced the problem on low yield with the reasons of low soil fertility, diseases and insect pests (Soytong, 1992). Moreover, the toxic chemical residues to tomato leading to harzadous effect to consumers due to over use chemical pesticides

* Corresponding author: Sibounnavong Phonesavard; e-mail: Kitoy_lk@hotmail.com

(Cowell, 1979; Villareal and Lai, 1978). There are some reports on food safety production that has been proposed the methods of good agricultural practice (GAP), pesticide-free production (PFP) and organic crop production (Sibounnavong *et al*., 2006). With this, GAP concerns on reduction of chemical pesiticides whereas PFP and organics concern on termination of chemical pesticides and use the biological products (Tann *et al*., 2011; Sibounnavong *et al.,* 2011). Biological control of diseases and insects become increasingly promotion to be used for crop production in many countries (Soytong *et al.,* 2001). Soytong (2004) reported on microbial products for bio-agriculture have been released to the growers over 15 years to promote GAP, PEP and organic crop production. These bio-products include bio-organic fertilizers (bio-fertilizers), bio-humus, liquid microbial fertilizer, biofungicide (*Chaetomium* sp.) and bioinsecticide (*Metarhizium* sp. and *Beauveria* sp), etc. *Chaetomium* is commercialized bio-fungicide mixing 22-strains of *Chaetomium cupreum*and *Chaetomium globosum* which *C. cupreum* strain CC003 found to produce rotiorinol (Kanokmedhakul*et al.,* 2006) and *C. globosum* produces chaetoglobosin-c (Kanokhamedhakul*et al*., 2001) as control mechnism to inhibit several plant pathogens e.g. *Phytophthora* spp., *Pythium* spp. (Pornsuriya *et al.,* 2008), and *Fusarium* spp (Soytong *et al*., 1992). Successful application of Chaetomium bio-fungicide in the fields have been demonstrated in several countries e.g. Thailand, P.R. China, Costa Rica, Vietnam, Laos, Philippines, Bangladesh, Cambodia, Georgia and Russia (Soytong, 1992; Shternshis *et al*., 2005; Kaewchai *et. al*., 2009 and Kean *et al*., 2010).

Mycephyt is a natural plant growth stimulator represents biologically active, naturally balanced complex, prepared from the growth medium of the mycorhizal fungi. Mycephyt application is effective both outside and in greenhouses. Mycephyt is safe for humans and environment: non-toxic; non-mutagenic; does not irritate skin and eyes (Tann *et al.,* 2011). The preliminary test of formulated combination of Chaetomium bio-fungicide and Mycephyt namely CM product was tested in tomato that gave very good result (Soytong, *et al*., 2010). Moreover, Sibounnavong, *et al.* (2010) reported that the new formulation of bio-product namely CM product (combination of Chaetomium bio-fungicide and Mycephyt-plant growth stimulant) had been successfully tested with rice, kale, kangkong, coriander. The objective of research project was to evaluate agricultural inputs used for good agricultural practice (GAP), pesticide-free production (PFP) and organic methods.

Materials and methods

Morphology of Chaetomium spp.

Pure cultures of *Chaetomium cupreum* CC03 and *Chaetomium globosum* Cg 05 were offered by Associate Professor Dr. Kasem Soytong, KMITL, Bangkok, Thailand. The cultures were then transferred to potato dextrose agar (PDA) and incubated at room temperature (27-30 C) for periodically observation for 3 weeks. Characteristics of fungi were observed under binocular compound microscope.

Morphology of Fusarium oxysarum f sp lycopersici NSKCO02

The most virulent isolate of *Fusarium oxysarum* f. sp. *lycopersici* NSKCO02 causing tomato wilt var Sida was offered by Dr. Phoutthasone Sibounnavong, National University of Laos, Lao P.D.R. Pure culture was subcultured to PDA and incubated at room temperature (27-30 C) for 7 days or until grown in full plate. Characteristics of fungus were observed under binocular compound microscope.

Effect of Mycephyt for the growth of C. cupreum, C. globosum and F. oxysarum f splycopersici

The Mycephyt is secondary metabolites from endophytic fungus in the form of white soluble powder. It was tested to determine its effect for the growth of *C. cupreum*, *C. globosum* and *F.oxysarum* f. sp *lycopersici* NSKCO02. The experiment was conducted by using Completely Randomized Design (CRD) with 4 replications. The different concentrations (treatments) of Mycephyt were tested at the concentration of 0, 10, 50, 100, 500 and 1,000 µg/ml. Each concentration was amended into PDA and autoclaved at 121°C, 15 psi for 20 minutes, and poured into sterilized Petri dishes about 5 ml/plate. The culture of test fungi was transferred onto the test plate amended with Mycephyt in each concentration by cutting agar discs from margin of actively growing colony by sterilized cork borer. The culture agar disc was placed at the center of Petri dishes in each concentration and incubated and room temperature (27-30 C). The colony diameter (cm) was measured at 2 days intervals until the fully in control plates. The spores were counted by using Haemacytometer.

Efficacy test of good agricultural practice (GAP), pesticide-free production and organic agriculture for the growth of tomato varieties

The experiment was conducted by using two factors factorial experiment in Randomized CompletelyBlock Design (RCBD) with 4 replications. Factor A represented three varieties of tomato; A1: Cherry Variety; A2: Loukthor Variety and A3: Sida Variety. Factor B represented different application methods; B1: Non-treated control, B2: Organic mrthod, B3:Pesticide-free production (PFP), B4: Good agriculture practices (GAP) and B5; chemical method. The 15 day old seedlings of tomato var. cherry, loukthor and sida were used. The soil mixture of soil:compost at the rate of 10:1 v/v was sterilized at 121 C for 1 h in two consecutive days. The sterilized soil was put into clay plot (12 inch dia) before experiment. The non-treated control was only applied water and weeding throughout the experiment. The organic method was applied municipal compost at the rate of 5 g/pot (12 inches diameter) before planting, then spayed liquid biofertilizer 40 cc/20L, Bioinsecticide (*Metarhizium* and *Beauveria*) 40 cc/20L, and CM product 10 g/20L at every 15 days until harvest. The pesticide-free production (PFP) was applied chemical biofertilizer (9-3-4) at 5 g/pot before planting, and applied chemical biofertilizer (8-3-8) at 5 g/pot after flowering, then spayed liquid biofertilizer 40 cc/20L, Bioinsecticide (*Metarhizium* and *Beauveria*) 40 cc/20L, and CM product 10 g/20L at every 15 days until harvest. GAP method (50 % chemicals and 50 % biological applications) was performed by applying chemical-biofertilizer (9-3-4) at the rate 5 g/pot after plantimg, and appied chemical biofertilizer (8-3-8) at the rate of 5 g/pot after flowering. Alternate spraying at 15 days until harvest with chemical pesticide (chemical insecticide, abamectin 20 cc/20L and chemical fungicide, benomyl 5 g/20L) and biolocial pesticide (liquid biofertilizer 40 cc/20L, Bioinsecticide (*Metarhizium* and *Beauveria*) 40 cc/20L, and CM product 10 g/20L). Chemical method was appled with urea (46-0-0) at the rate of 2 g/pot, and applied 15-15-15 at the rate of 2 g/pot after flowering, and spraying chemical pesticide at 15 days until harvest (chemical insecticide, abamectin 20 cc/20L and chemical fungicide, benomyl 5 g/20L).

Data were collected as plant height at every 20 days (cm), fresh weight of tomato (g), dry weight (g), root length (g), fresh weight root (g), dry weight root (g), number fruit, fruits weight (g) and size of fruit (cm).

Statistical Analysis

All data collceted were summarized and computed analysis of variance in factorial arrangement of Completely Randomized Design (CRD). Treatments

found significantly different were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and P=0.01.

Results and discussions

Chaetomium globosum Cg 05 and *Chaetomium cupreum* CC03 and were morphologically studied their characteristics under compound microscope. These isolates are used to formulate the bio-fungicide for plant disease control (Soytong *et al.,* 2001).

Chaetomium globosum Kunze

The growth rate of colony on poatato dextraose agar (PDA) reaches full plate of 9 cm within 7-10 days with pale aerial mycelium and often with grey green; ascomata maturing within 10 days, olivaceous or brown in reflected light, superficial, ovate with ostiolate, 170-250 µm;, terminal hairs numerous, usually unbranched, flexuous, undulate or slightly coiled, tapering, septate, brown, asci clavate, stalked, 30-40 x 10-15 µm, 8-spored, evanescent; ascospores limoniform, brownish when mature, thick-walled, 8-12 x 6-8 µm, with an apical germ pore. The isolate was also similar reported by Soytong and Quimio,1989; Soytong, 1991; Pornsuriya *et al.* (2008). It is interested that this isolate of *C. globosum* Cg 5 is reported to be antagonized many plant pathogens (Soytong *et al.,* 2001) and it could produce antiviotic substances namely chaetogobosin-C to suppress some plant pathogens (Kanokmedhakul *et al.*, 2001) and induce immnunity to tomato against Fusarium wilt disease (Chareonporn *et al.*, 2010).

Chaetomium cupreum Ames

Colony on PDA are pinkish shade and reddish pigment exudated. Ascomata maturing within 15 days, ovate, 80-140 x 94-100 µm. Terminal hair arcuate, apically circinate or coiled, septate. Ascus is clavate with 8 ascospores. Ascospores are reniform, 4.5-6.0 x 6.5-9.0 µm, with a single apical germ pore. This was similar characteristics with the reports of Soytong and Quimio, 1989; Soytong, 1991; Pornsuriya *et al.* (2008). Moreover, this isolate had been proved to produce antibiotic substances, e.g, rotiorin, rotiorinol A etc. (Kanokmedhakul *et al.*, 2001).

***Fusarium oxysporum* f sp *lycopersici* NSKC02**

F. oxysporum f sp.*lycopersici* NSKC02 grown on PDA showed whitish cream when young and turn pale purple when mature. It produced macroconidia with well-developed at the tip, 3-5 septate macroconidia and abundance microconidia with single or two cells. Chamydospores were observed as intercalary at maturity. This isolate NSKC02 was resported by Sibounnavong *et al.* (2008) as a virulent strain to cause tomato wilt var Sida.

Effect of Mycephyt for the growth of C. cupreum, C. globosum and F. oxysarum f splycopersici

Result showed that Mecyphyt at 1000 µg/ml gave significantly highest colony growth at 2 day incubation when compared to 0 µg/ml. The colony growth was not significantly different in colony growth at concentration of 10 - 500 µg/ml and control (0 µg/ml). Moreover, the colony growth at 4 days of the concentration of 100-1000 µg/ml was significantly better than the concentration of 10 µg/ml and differed from the control. After 4 day incubation, Mycephyt at concentrations of 10-1000 µg/ml was not significantly differed in colony growth and also not significant difference when compared to 0 µg/ml (Table 1). It revealed that Mycepht is not promoted or inhibited *C. cupreum* CC03. Mycephyt is reported to be a secondary metabolite from endopgytic fungus to stimulate plant growth (Sibounnavong *et al.*, 2011). The effect of Mycephyt on the growth of *C. globosum* at 2, 4 and 6 days was also shown to be no affected (Table 2). And Mycephyt was not inhibited *F. oxysporum* f sp.*lycopersici* NSKC02 causing tomato wilt at 6 days (Table 3). It is proved that Mycephyt have no affected on the colony growth of *C. globosum* Cg 05 and *C. cupreum* CC03 and *F. oxysporum* f sp *lycopersici* NSKC02. So, In this study, formulation of CM product from Chaetoium-biofungicide for plant disease control combination with Mycephyt for plant growth would be confirmed that those tested fungi had no affected on colony growth. It is indated that Mycephyt contains secondary metabolite could possible to inhibit thre growth of pathogen, *F. oxysporum* f sp *lycopersici* NSKC02.

Table 1. Colony diameter (cm) of *Chaetomium cupreum* at 2, 4 and 6 days

Mecyphyt, µg/ml	2 days	4 days	6 days
0	1.97 bc[1]	2.68 ab	4.66 a
10	2.06 ab	2.91 a	4.97 a
50	1.82 d	2.31 b	4.13 b
100	1.95 bc	3.05 a	4.97 a
500	1.92 cd	2.80 a	4.71 a
1000	2.13 a	3.07 a	5.00 a
C.V(%)	2.69	7.62	4.95

[1]Average of four replications. Means followed by a common letter are not significantly different by DMRT at P=0.01.

Table 2. Colony diameter (cm) of *C. globosum* at 2, 4 and 6 days

Mecyphyt, µg/ml	2 days	4 days	6 days
0	2.76 a[1]	4.55 b	5a
10	3.17 a	4.75 ab	5a
50	2.77 a	4.67 ab	5a
100	2.80 a	4.72 ab	5a
500	2.76 a	4.62 ab	5a
1000	3.02 a	4.87 a	5a
C.V(%)	9.41	2.85	-

[1]Average of four replications. Means followed by a common letter are not significantly different by DMRT at P=0.01.

Table 3. Colony diameter (cm) of *Fusarium oxysporum* at 2, 4 and 6 days

Mecyphyt, µg/ml	2 days	4 days	6 days
0	1.71 a[1]	4.01 ab	5.00 a
10	1.83 a	4.12 a	4.86 ab
50	1.87 a	4.11 a	4.83 abc
100	1.85 a	4.12 a	4.61 c
500	1.70 a	3.97 ab	4.63 bc
1000	1.72 a	3.88 b	4.60 c
C.V(%)	6.25	3.41	2.38

[1]Average of four replications. Means followed by a common letter are not significantly different by DMRT at P=0.01.

Result showed that Mycephyt promoted spore production of *C. cupreum* at the concentration of 100, 500 and 1000 µg/ml which the number of spores of 11.18, 13.56 and 26.56 x 10^6/ml but the lower concentration of 10-50 µg/ml were not significantly different when compared to the control (0 µg/ml). Result showed significanltly highest spore production of *C. globosum* at concentrations of 1000 µg/ml which number of spores of 82.18 x 10^6

spores/ml, and followed by the concentrations of 500 and 100 µg/ml which the number of spores were 63.06 and 47.99 x 10^6 spores/ml, respectively. It is indicated that Mycephyt simulated both of *C. cupreum* and *C. globosum* which act as biological fungicide for disease control. It is undoubledtly stated that Chaetomium-powder can be synergisted to Mycephyt which formulated as CM product as previus report by Sibounnavong *et al.*, 2011; Tann *et al.*, 2011). In the meantime, Mycephyt significantly inhibited the tested pathogen, *F. oxysporum* f sp. *lycopersici* significantly inhibited the conidia production at the concentration of 10-500 µg/ml, when compared to the control (0 µg/ml). Mycephyt at 1000 µg/ml gave the highest inhibition of spore production (81.75 x 10^6 spores/ml) when compared to the control (166.43 x 10^6 spores/ml) as seen in Table 4.

Table 4. Number spore of *C. cupreum, C. globosum* and *Fusariumoxysarum* f sp.*lycopersici*

Mecyphyt, µg/ml	***C. cupreum*** **(x 10^6/ml)**	***C. globosum*** **(x 10^6/ml)**	***F. oxysporum* f sp *lycopersici*** **(x 10^6/ml)**
0	9.50 bc[1]	48.43 bc	166.43 a
10	3.81 c	24.93 c	127.93 b
50	4.56 c	34.75 c	122.12 b
100	11.18 b	47.99 bc	131.50 b
500	13.56 b	63.06 ab	104.62 bc
1000	26.56 a	82.18 a	81.75 c
C.V(%)	25.27	23.55	12.45

[1]Average of four replications. Means followed by a common letter are not significantly different by DMRT at P=0.01.

Efficacy test of good agricultural practice (GAP), Pesticide-free production and Organic agriculture for the growth of tomato varieties

Comparison of GAP, PFP and organic methods in three varirties of tomato (Cherry, Loukthor and Sida) gave significantly better in growth parameters or plant stands than the non-treated control. At 20 days after treatments, it was not significantly differed in plant height of all application methods in Sida variety but there were significantly differed in Cheery and Loukthor varieties than the non-treated control. At 40 days, the organic method in Cherry variety gave the highest in plant height and followed by PFP, GAP and chemical method, respectively when compared to the non-treated control. In Loukthor variety at 40 days, there were not sifnificantly differed in plant height in organic, PFP and GAP but differed from chemical method. In Sida variety at 40 days showed that the organic method in Cherry variety gave the

highest in plant height and followed by PFP, GAP and chemical method, respectively. However, after 70 days of experiment showed that organic, PFP, GAP and chemical methods gave significanytly differed inj plant height than the non-treated control in all tested varities (Table 5 and Fig. 1).

Result showed that fresh and dried weight of stem, root length, fresh and dreid weight of roots in organic method was significantly better than PFP, GAP and chemical methods in all tested varietires when compared to the non-treated control. As a result, root length, fresh and dried weight of roots at 70 days in organic, PFP and GAP were significantly better than the non-treated control in all tested tomato varieties (Figs. 2 and 3). It is indicated that organic method with potent bio-products as CM product for disease control, bio-insecticide (*Beauveria* and *Metarhizium*), bio-fertilizer would promote plant growth parameters of tomato var Cherry, Loukthor and Sida. The organic method gave significantly highest number of fruits in all tested varieties, followed by PFP and GAP. But in chemical method gave lower number of fruits when compared to organic, PFP, GAP and non-treated one. It is obsereved that the chemnical method and non-treated control faced the wilt incidence caused *F. oxysporum* f sp *lycopersici* thereafter diagnosis and isolated the pathogen to identify. The organic method was applied municipal compost, liquid biofertilizer, Bioinsecticide (*Metarhizium* and *Beauveria*), and CM product at every 15 days until harvest proved that thease agricultural inputs can be used instead of chemical ones as reported by Sibounnavong *et al.* (2006). The pesticide-free production (PFP) applied chemical biofertilizer of 9-3-4 and 8-3-8, spayed liquid biofertilizer , Bioinsecticide (*Metarhizium* and *Beauveria*) and CM product at every 15 days until harvest without applying toxic chemical pesticide were also proved as reported by Sibounnavong *et al.* (2011)and Tann *et al.* (2011).

Table 5. Plant height of tomatoes after treatment for 20, 40 and 70 days

Varieties	Methods	20 days	40 days	70 days
Cherry	Control	40.25 bcd[1]	82.50 cd	87.25 bc[1]
	Organic	41.80 bcd	104.50 a	117.50 a
	PFP	37.67 cd	100.00 ab	113.75 a
	GAP	34.50 de	93.00 bc	95.50 b
	Chemical	29.50 e	76.87 def	111.75 a
Loukthor	Control	36.00 cde	62.00 gh	67.00de
	Organic	42.37 bc	68.75 fg	74.25 cde
	PFP	39.87 bcd	70.75 efg	82.25 bcd
	GAP	40.12 bcd	68.25 fg	82.50 bcd
	Chemical	38.75 cd	48.50 i	85.25 bc
Sida	Control	47.22 b	61.37 gh	63.75 e
	Organic	55.37 a	80.12 de	82.50 bcd
	PFP	47.37 b	66.00 fg	67.50 de
	GAP	46.50 b	65.75 fg	71.75 cde
	Chemical	47.22 b	54.12 hi	76.00 cde
	CV (%)	8.53	7.49	9.05

[1]Average of four replications. Means followed by a common letter are not significantly different by DMRT at P=0.01.

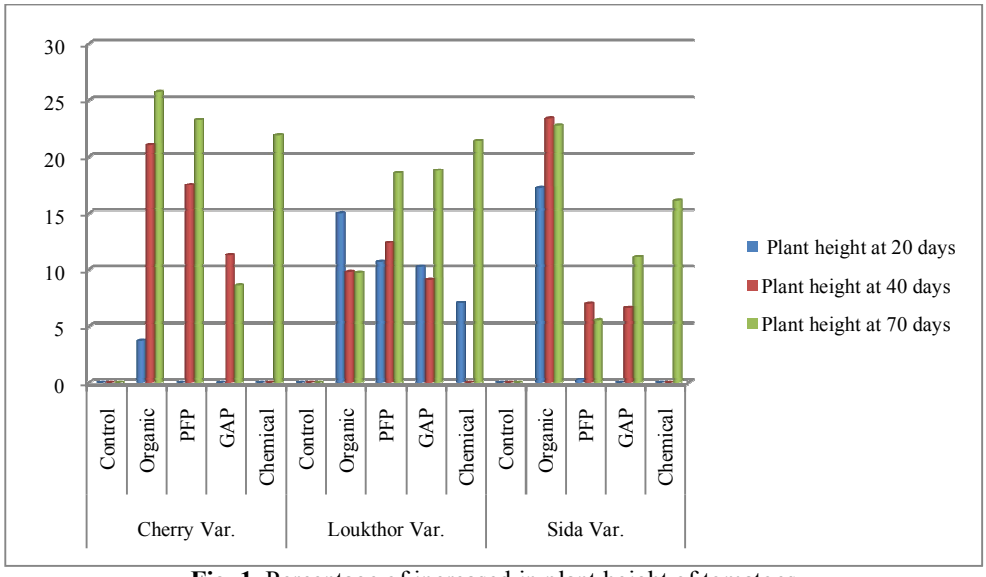

Fig. 1. Percentage of increased in plant height of tomatoes

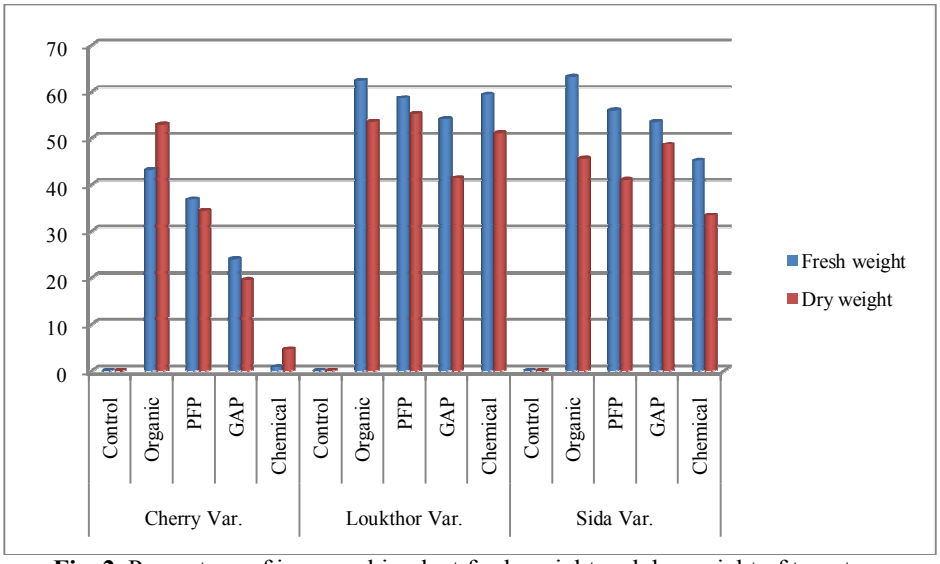

Fig. 2. Percentage of increased in plant fresh weight and dry weight of tomatoes

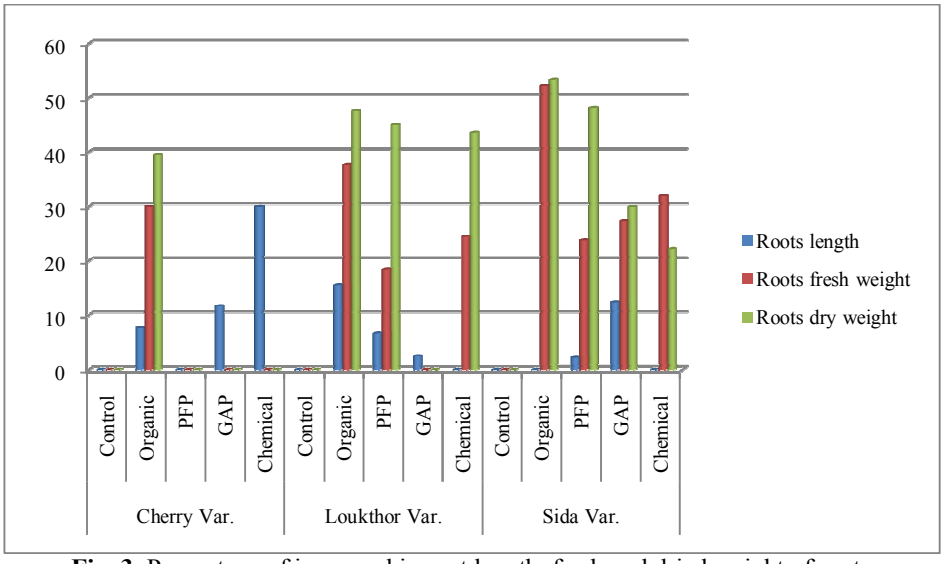

Fig. 3. Percentage of increased in root length, fresh and dried weight of roots

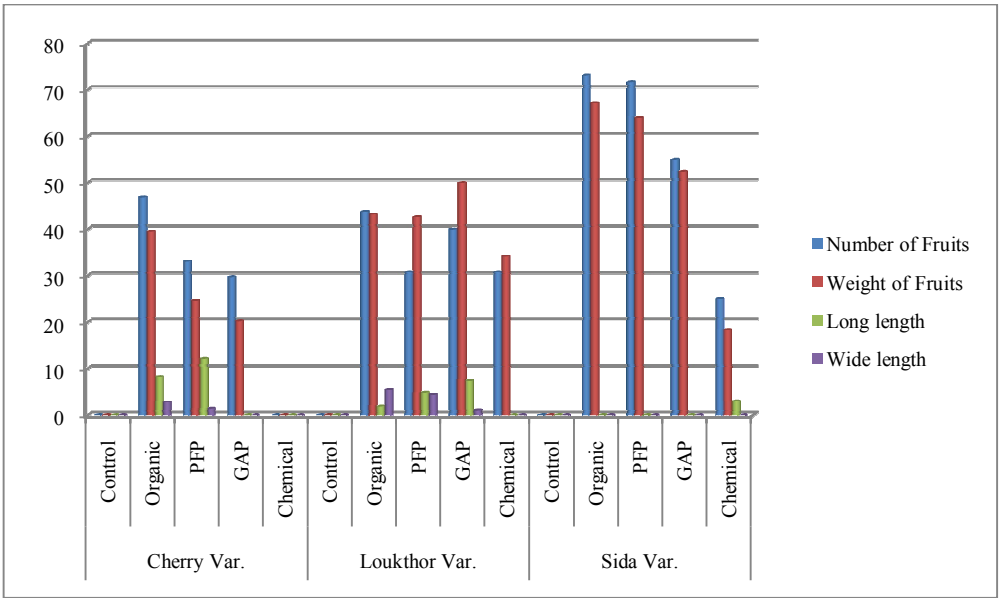

Fig. 4. Percentage of increased in parameters of fruits

It is concluded that in Cherry variety, the organic method increased in plant height, fresh and dried weight of plant of 25.74, 43.08 and 52.87 %, respectively. PFP method increased in plant height, fresh and dried weght of plant of 23.29, 36.78 and 34.40 %, respectively. GAP method increased in plant height, fresh and dried weight of plant of 8.63, 24.03 and 19.60 %, respectively. Result in Loukthor and Sida varieties were also increased those plant growth parameters. This similar result reported by Sibounnavong *et al.,* 2011) who worked on organic kales, kangkong (Sibounnavong *et al.,* 2006). It was shown that Cherry variety in organic method increased frult number of 46 % while PFP and GAP increased 33 and 29 %, respectively but chemical method had no increased in fruit number (Fig.4). It was also revealed that Lourthor variety in organic method increased frult number of 43 % while PFP, GAP and chemical methods increased 30, 40 and 30 %, respectively. Sida variety in organic method increased frult number of 73 % while PFP, GAP and chemical methods increased 71, 55 and 25 %, respectively. It was observed that Sida variety in non-treated control seriously infected and showed wilt symptom caused by *F. oxysporum* f sp *lycopersici* and low yield or fruit number as Sibounnavong *et al.*, 2008; Soytong, 1992) who stated that tomato wilt caused by *F. oxysporum* f sp *lycopersici* leading to yield loss over 60-80 %. The result is also confirmed that the agricultural in puts of Chaetomium plus Mycephyt as CM product as biofungicide and growth stimulant (Kwaechai *et al.,* 2009), bioisecticide (*Beauveria and Metarhizium*), liquid biofertilizer can be successfully used for

organic, PFP and GAP, or even used with chemical method by alternative application.

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