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Finding natural alternatives to methyl bromide in greenhouse cantaloupe for yield, quality and disease control

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Abstract: To find natural alternatives to methyl bromide for reducing its environmental damage, a greenhouse study was conducted in grown cantaloupe plants (*Cucumis melo* L) cv Primal F1 Hybrid. Nine treatments were evaluated to find a better natural methyl bromide (MBr) alternative which are, Bio-fumigants (with Fresh cow manure), Bio-fumigants (with Fresh chicken manure), Brassicaceae residuals treatment, Cultivation in local materials using rice straw bales, adding disease Suppressive Compost (Bio-Compost), using hot water for killing soil pathogens, Biological control by using of *Trichoderma harzianum* and *Bacillus subtilis* in comparison with chemical control with one of MBr chemical alternatives (Basamid) and Control (farmer treatment). Best results in seedlings survival rate and wilt disease incidence (%) was recorded with the use of Basamid but it was also noted that good results were obtained by Brassicaceae residuals treatment, followed by bio-fumigation with Fresh cow manure, in comparison to the control treatment (Farmer treatment). Bio fumigation with fresh cow or chicken manure followed by Brassicaceae residuals and lastly Bio Compost resulted in a significant increase in soil temperature during the fermentation period. On the other hand, hot water treatment had increased soil temperature markedly and rapidly but it had been lost quickly. Regarding yield characteristics, chemical control treatment (Basamid) resulted in the highest yield parameters followed by biological control treatment, Bio compost, hot water injection, and fresh cow manure with no significant difference among them. While fresh chicken manure and Brassicaceae residuals had a slight significant improvement in yield parameters than farmer treatment. On the other hand, it was noticed that cultivation in rice straw was not very successful in giving the desired plant yield and had the lowest values in all yield and quality components which may be due the large canopy size of cantaloupe plants.
Keywords: Bio-fumigants, Bio-Compost, Brassicaceae residuals, biological control, hot water treatment, Basamid, rice straw.

Introduction

In Egypt, vegetables are considered the most high value cash crops for growers and also, it is an important source of hard currency for the country. In summer season, they are grown under field conditions, but in winter and off-season production, they are grown under plastic houses. Under greenhouse growing condition, it is a favorable condition for pests and diseases so farmers are used to use high amounts of highly toxic pesticides to protect their investments. Methyl bromide (MBr) is one of these toxic chemicals which greenhouse growers are used to use to disinfect their fields prior transplanting cantaloupe. Under the Clean Air Act and

Montreal Protocol, production of methyl bromide, a widely used fumigant in agriculture and forestry that is also an ozone-depleting substance, was phased out since 2005 but there are allowable exemptions for users who do not have technically or economically feasible alternatives, According to environmental laws, no methyl bromide are legally produced any more, consequently no MBr will be available within the coming years.

In the absence of soil fumigation with methyl bromide, due to its damaging effect on the environment, there is an urgent need for new alternatives and preferably natural alternatives. These new alternatives needs to be evaluated for efficacy to provide a short-term solution for control of soil-borne pathogens. The use of organic amendments (OAs) to improve soil properties, plant health and yield has expanded in recent years[1]. In this regards, several researchers have been recorded that bio compost formulated with bio-control agents application as soil amendments could suppress diseases caused by *Rhizoctonia solani* and *Fusarium spp.* **Bullock and Ristaino (2002)**[2] mentioned that using composted cotton-gin trash to control southern blight on tomatoes resulted in reduction of the disease incidence and also enhanced population of beneficial soil microbes. **Abd-El Moity (2001)**[3] recommended a combination between polyethylene mulch solarization, organic amendments, sulfur and a group of biocontrol agents to control different diseases and other pests including nematodes and weeds in the field. using organic soil amendments as shredded broccoli plants alone or with high rates of compost and VAM (Vesicular –Arbascular Mycrohizae) was not sufficient to maintain roots pathogen free season long[4]. Application of compost and compost extract to suppress damping off and some other foliar diseases were efficient [5]. Amendments of cruciferous residues have been extensively studied for their potential as OAs[6, 7]. In earlier reports, it was declared that solar heating of soil amended with cabbage residues eliminates *Fusarium oxysporum* in closed containers under laboratory conditions[8]. On the other hand, solarization of soil amended with chicken compost effectively controlled *Meloidogyne incognita* and *Pythium ultimum* Trow[9].

In Egypt, the annual production of rice straw reached more than 5 million tons, which causes serious pollution when disposed by burning. On the other hand, the major components of rice straw are not favorable for soil fungi or nematodes.

The use of compacted rice straw bales as organic medium and an alternative for methyl bromide is a new approach in Egypt. It was reported that rice straw as an alternative substrate in plastic house gave the highest yield and improved fruit quality of sweet pepper[10]. **Jarvis (1997)**[11] reported that wheat straw bales have traditionally been used for cucumber and tomato production in the United Kingdom.

The aim of this work is to find out a new method to replace MBr in greenhouse production of cantaloupe with a natural or biological method. This method must be effective as MBr in controlling different pathogens and parasites. The present work used Bio-fumigants, Brassicaceae residuals, Cultivation in local materials using rice straw bales, disease Suppressive Compost, soil boiling water injection, Biological control in comparison with chemical control with one of MBr chemical alternatives (Basamid) and Control (farmer treatment) in order to find natural or environmentally safe alternatives to methyl bromide to reduce the environmental damage resulting from it.

Materials and Methods

The present work was conducted in a greenhouse at the experimental station of the national research Center in Nubaria region (Behira governorate), aiming to find natural alternatives to methyl bromide to reduce its environmental damage in greenhouse grown cantaloupe plants. The experimental soil had a sandy texture with pH of 7.6, EC-value of 0.81(dS m^{-1}in soil paste) and the organic matter was 0.19% with 15.00, 9.40, 16.00 mg/100g soil contents of N, P, and K respectively.

Plant material and treatments

Seedlings of cantaloupe (*Cucumis melo* L) cv Primal F1 Hybrid. Were transplanted in the greenhouse in the second week of February 2011 and 2012 using standard plasticulture practices. Beds were 20 cm high and 72 cm wide, containing 2 rows of plants spaced 30 cm apart within, and between rows with beds spaced 1.4 m on center. A randomized complete block design with four replications per treatment was used; each of three adjacent beds 4 m long x 4.2 m wide. Treatments studied were Bio-fumigants (with Fresh cow manure), Bio-fumigants (with Fresh chicken manure), Brassicaceae residuals treatment, Cultivation in local materials using rice straw bales, adding disease Suppressive Compost (Bio-Compost), using hot water for killing soil pathogens, Biological control by using of *Trichoderma harzianum* and *Bacillus subtilis* in comparison with chemical control with one of MBr chemical alternatives (Basamid) and Control (farmer treatment).Irrigation and nutrient supply was provided via a drip system. Fertigation and pest control were followed as commonly practiced for cantaloupe production under unheated plastic house conditions.

The different treatments were carried out as follows:

1. Bio-fumigation using fresh cow manure

Based on the use of gases from the biodegradation of organic matter such as fresh cow manure. Fresh cow manure and sulfur were mixed with soil using mechanical tractor. The treated soil was gently sprayed with water after adding and mixing the manure and installing the drip irrigation system. The treated soil was then covered with Polyethylene sheets 100 µ. Soil was irrigated three times a week to keep fermentation process active. Polyethylene sheets were maintained against any damage to keep all produced gases beneath polyethylene sheets to allow all the produced gases to be accumulated and reach lethal dose. Polyethylene sheets were maintained for 3 weeks then soil was heavily irrigated before cultivation.

2. Bio-fumigation using fresh chicken manure

Similar to the previous treatment except using fresh chicken manure.

3. Cultivation on substrates (Rice Straw)

Compacted rice straw bales (50 cm height x 70 cm width x120 cm length)obtained from commercial suppliers were arranged to form a row and were placed on a sheet of plastic film to isolate the straw from the soil. Drip irrigation lines were laid on top of the rice straw bales with two driplines on each row installed at a distance of 40 cm. For rice straw fermentation and to optimize C/N ratio to the suitable levels for plant growth ,the straw bales were first irrigated for six hours for washing out soil particles, and then the dissolved ammonium and potassium sulphate and phosphoric acid 85% fertilizers were applied daily through the irrigation system for two weeks before planting.

4. Boiling water injection into the soil

Hot water injection was done by boiling water in barrel and water temperature was checked and then soil was injected with the boiled water through the drip irrigation system which is under 100µ black polyethylene mulch. Soil temperature was checked to assure that the temperature reached at least 70°C and it must be achieved for at least 30 minutes before terminating the treatment. Treatment was repeated for 3 consecutive days before transplanting day.

5. Plant extracts using Brassicaceae residuals treatment

Manure and sulfur were add to the line then fresh shredded Brassicaceae residuals were added at 5 kg/m^2 and they were mixed together with soil using mechanical tractor. The Mix were covered with soil, drip irrigation lines were laid out and finally beds were covered with 100µ black polyethylene mulch. It was irrigated three times a week to keep fermentation process active. Polyethylene sheets were maintained for 3 weeks then soil was heavily irrigated before cultivation.

6. Disease suppressive compost (Bio-compost)

Using Compost of bagasse inoculated with effective microorganisms which suppresses disease and enriches soil with nutrients. This compost was prepared by plant pathology lab, National Research Center. Disease Suppressive Compost were added at 7 kg/m^2 and covering with plastic mulch for 3 weeks before planting.

7. Bio-control

Use of bio-control agents was as transplant drench prior cultivation a mix of beneficial micro-organisms namely *Trichoderma harzianum* and *Bacillus subtilis*.

8. Chemical alternative treatment(Basamid)

After adding compost, Basamid 97 GR (BASF, Germany) a commercial product containing 97% of dazomet, was spread at a concentration of 50 g/m^2 Mixed with soil and sprayed with a small amount of water with the hose (enough to get the Basamid wet) and covered immediately with plastic mulch. Treatment was done 3 weeks prior to planting each year.

9. Farmer treatment

By using only farmer treatment without any application of soil sterilization (as a Check)

Sampling and Data Recording:

1. Soil temperature monitoring

Soil temperature in treatments with expected to have an effect on rhizosphere temperature (Fresh cow manure, Fresh chicken manure, Brassicaceae residuals, Bio Compost, and Hot water injection) was mentored and recorded twice weekly or a month before planting (just after starting these treatments). Regarding Hot water treatment, soil temperature was recorded daily till planting day (4 total readings)

2. Biological data

The percent of survival rate and fusarial wilt disease incidence of seedlings were calculated after 15 days from transplanting.

3. Vegetative growth

Three plants from each replicate were chosen randomly at 30 days after transplanting and the following data were recorded: Plant height (cm), number of leaves/plant, number of branches/plant, fresh and dry weights of leaves/plant (g).

4. Fruit yield and quality

Ten randomly selected cantaloupe fruits at marketable stage were harvested when the fruit reached at least the “half-slip” and the following data were recorded: fruit diameter, flesh thickness, no of fruits per plant, and average fruit weight, total weight of fruits in each plot was recorded and then the total fruit yield/m^2 was calculated.

5. Fruit chemical content

Random samples of three harvested fruits from each replicate at full ripening stage were chosen to measure organoleptic analysis where total soluble solids percentage (using hand refractometer), pH (using hand held pH meter) where measured. Also, titratable acidity were determined as mg/100g fresh weight according to **A.O.A.C. (1990)**[12]**.**

Experimental Design and Statistical Analysis

The experiment was arranged in a complete randomized plot design with four replicates, treatments were statistically analyzed and means separation was carried out using least significant difference (LSD) at $P<0.05$ according to the method described by **Gomez and Gomez (1984)**[13]. Combined analysis of the two seasons was carried out.

Results

Effect of different treatments on soil temperature in rhizosphere zone

As it clear from Fig. (1) that some of methyl bromide alternatives, such as Bio fumigation with cow or chicken fresh manure followed by Brassicaceae residuals treatment and lastly Bio Compost resulted in a marked increase in soil temperature during the fermentation and then dropped again to the normal level before cultivation while treatments which require no fermentation process didn't give any significant increase in soil temperature On the other hand, hot water treatment (treatment was done and repeated for 3 consecutive days prior to transplanting) have increased soil temperature markedly and rapidly but it had been lost quickly.

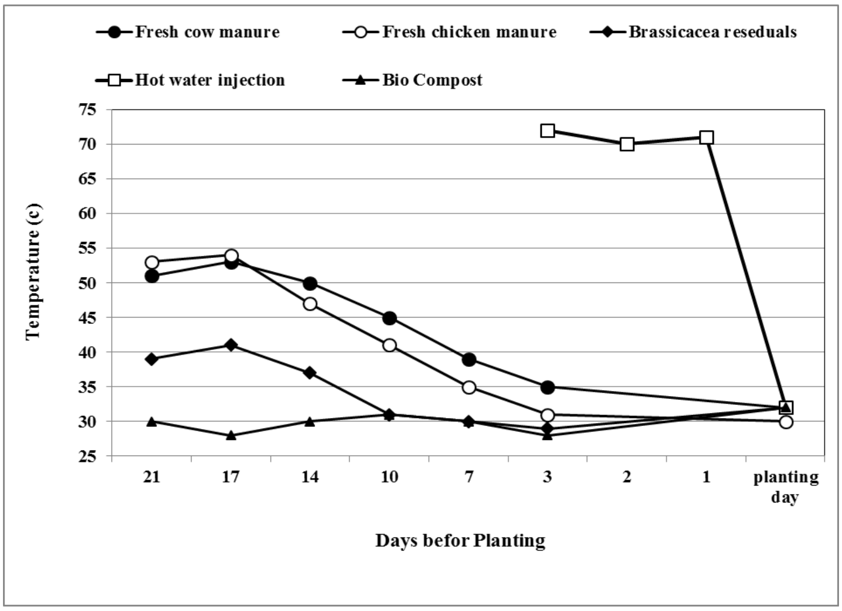

Fig. (1): Soil temperature in rhizosphere zone during treatment until planting day as affected by some methyl bromide alternatives.

Seedlings survival and vegetative growth

As shown from the results described in Fig. (2), seedlings survival rate and fusarial wilt disease incidence (%) recorded the best results with the use of chemical sterilization (Basamid) but also noted that significant results were obtained by Brassicaceae residuals treatment, followed by bio-fumigation with Fresh cow manure, fresh chicken manure and bio-compost respectively, in comparison to the control treatment (Farmer treatment).

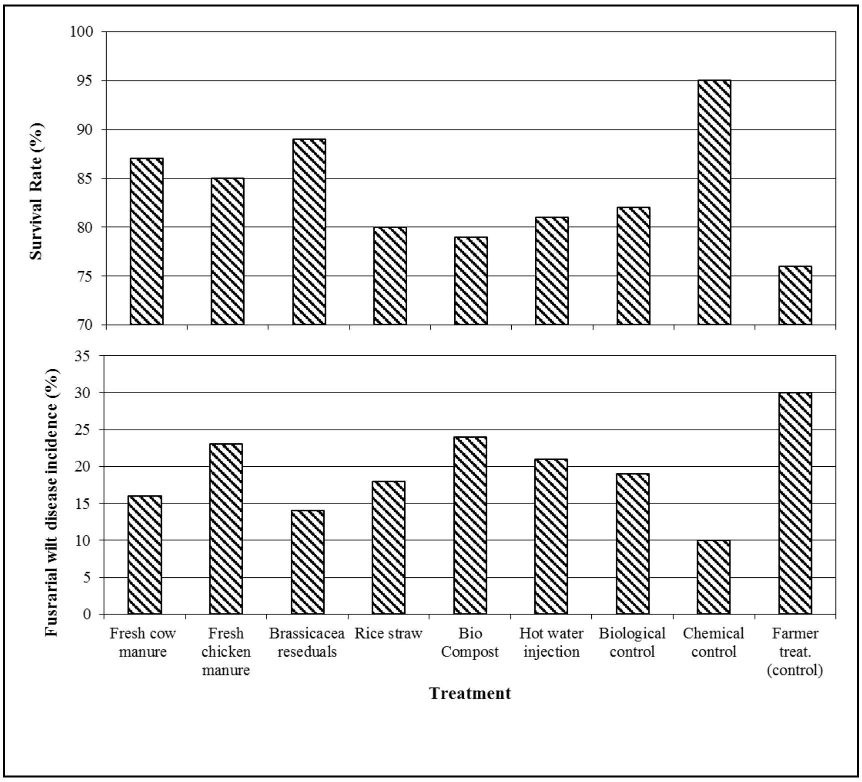

Fig. (2): The influence of soil treatment with some methyl bromide alternatives on survival rate and wilt disease incidence (%) in cantaloupe.

As for the effect of different methyl bromide alternatives on the vegetative growth characteristics, it was clear from the date illustrated in table (1) that chemical sterilization (Basamid) was the best plant vegetative growth but according to the MBr alternative used, bio-fumigation with fresh cow manure, fresh chicken manure, Brassicaceae residuals treatment and bio-compost respectively were the best and had a significant effect on plant vegetative growth, in comparison to the other treatments or control treatment (Farmer treatment).

Table (1): Effect of methyl bromide alternatives on the vegetative growth characteristics of cantaloupe plants.

Treatment	**Plant length (cm)**	**No. of leaves /plant**	**Plant fresh weight (g)**	**Plant dry weight (g)**
Fresh cow manure	87.76	65.6	89.63	8.26
Fresh chicken manure	85.12	66.7	91.18	9.99
Brassicacea residuals	83.28	64.1	90.01	9.98
Rice straw	78.88	52.4	75.32	7.59
Bio Compost	76.96	57.3	80.02	8.65
Hot water injection	62.72	43.9	63.36	6.23
Biological control	77.76	56.2	81.97	7.91
Chemical control (Basamid)	93.84	73.5	101.64	10.99
Farmer treatment (control)	69.28	49.8	71.23	7.98
LSD at 5%	3.36	5.1	13.33	1.26

Table (2): Effect of methyl bromide alternatives on fruit yield and fruit quality characteristics of cantaloupe plants.

Treatment	**Fruit weight (g)**	**Fruit diameter (cm)**	**No. of fruits/plant**	**Total fruit yield (Kg/m^2)**	**Flesh thickness (cm)**
Fresh cow manure	655	9.4	7.06	13.88	3.13
Fresh chicken manure	653	9.5	7.23	14.16	3.21
Brassicacea residuals	650	9.2	7.17	13.58	3.11
Rice straw	526	8.6	6.62	10.45	2.97
Bio Compost	621	8.9	6.70	12.48	2.78
Hot water injection	448	8.1	7.20	9.68	2.68
Biological control	628	8.8	7.25	13.15	3.16
Chemical control (Basamid)	677	9.7	7.72	15.67	3.45
Farmer treat. (control)	499	8.4	7.65	11.45	2.95
LSD at 5%	18.7	NS	NS	1.05	0.19

Fruit yield

As shown from data presented in Table (2), chemical control treatment (Basamid) resulted in the highest fruit yield parameters followed by biological control treatment, Bio compost, hot water injection, and fresh cow manure with no significant difference among them. While fresh chicken manure and Brassicaceae residuals had a significant improvement in yield parameters than farmer treatment. The highest fruit yield as total fruit yield / m^2 was obtained with fresh chicken manure while the using of bio-fumigation with fresh cow manure was the highest average fruit weight followed by cultivation on rice straw bales with no significant difference between them. On the other hand, the lowest fruit yield was obtained by biological control treatment followed by Brassicaceae residuals treatment. On the other hand, it was noticed that cultivation in rice straw was not very successful in giving the desired plant yield and had the lowest values in all yield components.

Fruit quality characteristics

As shown from data presented in Table (2), and Fig. (3) there were a clear effect of different methyl bromide alternatives on fruit quality of melon expressed as flesh thickness, total soluble solids (%), pH and titratable acidity. The best results were obtained by using bio-fumigation using fresh chicken manure followed by the using of bio-fumigation with fresh cow manure as compared with farmer treatment which recorded the lowest fruit quality values. On the other hand, chemical alternative gave a higher values of fruit quality parameters when compared with the other used natural alternatives

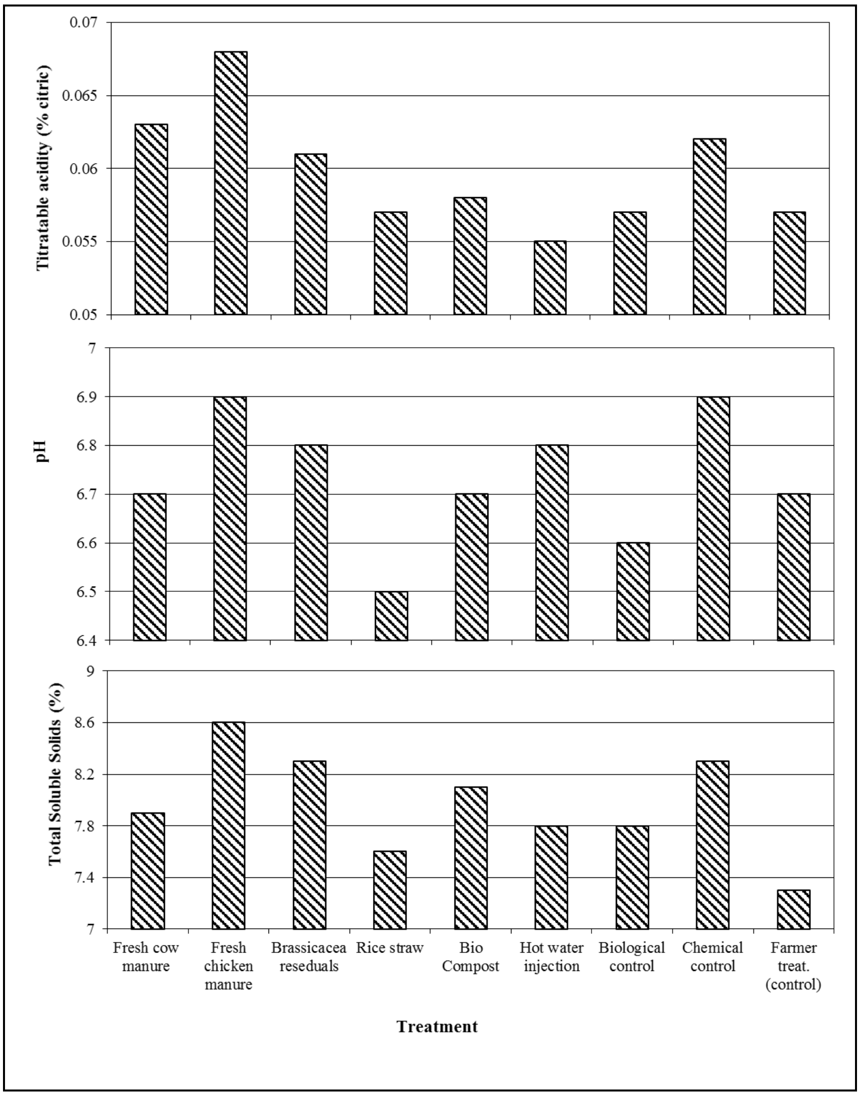

Fig. (3): The influence of soil treatment with some methyl bromide alternatives on titratable acidity (%), pH and TSS (%) in cantaloupe grown in sandy soil.

Discussion

It was clear from our investigation that Biofumigation has a similar efficiency as conventional pesticides for controlling vegetable soil pathogens, with the additional benefit of improving soil and plant qualities. The methodology required to put bio-fumigation into practice under commercial field conditions is approachable for both technicians and farmers. Its success is enhanced when included as part of an integrated management production system. We consider that the obtained results show that there are effective natural alternatives to MB. Bio-fumigation with fresh or cow manure, due to its efficiency and low cost, could be becoming the principal alternative practice in different productive areas in Egypt. Organic amendments play an important role as environmentally friendly and sustainable alternative approach to protect plants against soil borne pathogens. Soil amendments, using composted agricultural wastes fortified with bio control agents could be acceptable approaches in this regard. The beneficial effect obtained from amended soil with organic materials formulated with bio-control agents may be attributed to the activity increase of indigenous micro flora which resulting in suppression of pathogens population through competition or specific inhibition. The Application of Plant residues as crucifers, compost or manures rich in nitrogen, and other wastes may generate biologically active products in the soil which control plant pathogens[6, 14]. For example, amendments of cruciferous residues have been extensively studied for their potential as organic amendments[7]. Cultivation in local materials using rice straw bales, could reduce the risk of plant diseases, as well as benefit from the rice straw and reduces the environmental damage caused by burning it. Cucumber plants grown in rice straw bales under greenhouse conditions showed better growth and increased fruit number and weight compared with those grown in natural soil[15]. Strawberry plants growing in rice straw bales under open field conditions showed better growth and an increase in shoot and root systems[16]. Moreover, there was a slight increase in the total soluble solids (T.S.S) content in fruits strawberries grown in rice straw bales as compared with those of natural soil[16].

In our studied investigation, Adding disease Suppressive Compost (Bio-Compost) and biological control by using of *Trichoderma harzianum* and *Bacillus subtilis* were less effective than bio-fumigation with fresh cow or chicken manure although there are many reported investigations demonstrating that several composts and/or composts fortified with bio control agent used as soil amendments reduced pathogens density and protected plants from soil borne pathogens[17]. It is necessary to bring forward the date of MB elimination in order to reduce its deleterious impact on farmer and worker health and on the environment.

Conclusion

Results of this study demonstrate that production practices developed to provide alternatives to methyl bromide pre-plant soil disinfection can have significant impacts on cantaloupe product quality.

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