BIBLIOGRAPHY

CATHERINE APALING BAGSAN. NOVEMBER 2006. Management of Fusarium Wilt (Fusarium oxysporum f. sp. chrysanthemi) in Chrysanthemum (Dendranthema grandiflora T. Zveler) Using Bacterial Antagonists and Plant Extracts. Benguet State University, La Trinidad, Benguet.

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ABSTRACT

Selected bacterial antagonists and plant extracts were evaluated as possible alternatives to synthetic fungicides in managing *F*usarium wilt disease in chrysanthemum caused by *Fusarium oxyxporum f. sp. chrysanthemi* under the laboratory and greenhouse conditions. The study was conducted at the Biological Control Laboratory and greenhouse at Benguet State University, La Trinidad, Benguet from November, 2005 to October, 2006.

Bioassay test of the two bacterial antagonists *Flavobacterium* sp. and *Pseudomonas* sp. inhibited the growth of the pathogen *F. oxysporum* f. sp. *chrysanthemi* in culture forming inhibition zones. Among the plant extracts tested, garlic extract (*Allium sativum* L) significantly suppressed the growth of *F. oxysporum* f. sp. *chrysanthem*i in culture and gave the widest inhibition zone. Among the fungicides tested against *F. oxysporum* f. sp. *chrysanthemi,* mancozeb (Parafungus) gave significantly the widest zone of inhibition followed closely by benomyl (Benlate) , thiophanate methyl (Fungitox), thiophanate methyl (Topsin-M) and captan (Captan).

Chlorothalonil (Daconil) and mancozeb (Dithane M-45) did not inhibit the growth of the pathogen.

The test conducted to determine the compatibility of plant extracts and fungicides with the two bacterial antagonists, garlic extract and mancozeb showed incompatibility with *Flavobacterium* sp. and *Pseudomonas* sp. On the other hand, thiophanate methyl, gawed extract and table salt (NaCl) did not inhibit the growth of the bacterial antagonists. Therefore, in an integrated disease management program against *Fusarium* wilt of chrysanthemum mancozeb and garlic extract should not be combined with the biocon agents because these combinations will kill the beneficial bacteria.

Results of the greenhouse experiment conducted to determine the effectiveness of bacterial antagonists and plant extracts in reducing the soil population of *F. oxysporum* f. sp. *chrysanthemi* showed that the combination of *Flavobacterium* sp and *Pseudomonas* sp. was comparable with the standard fungicide, mancozeb. Although not as effective as the other treatments, application of garlic extract, Pseu*domonas* sp and *Flavobacterium* sp. alone and table salt significantly reduced the soil population of the pathogen compared with the untreated - inoculated plants. On the other hand, gawed extract was inferior with the other treatments in suppressing the pathogen population.

On disease severity, the lowest wilt infection was observed in plants treated with the fungicides, mancozeb and thiophanate. The result was consistent throughout the duration of the experiment. Although lower in efficacy, the combination of *Flavobacterium* sp. and *Pseudomonas* sp. and garlic extract significantly reduced wilt infection compared with the untreated/uninoculated plants. The highest wilt infection was observed in plants treated with hot water (applied before planting) + garlic extract

(added after 1 week) + combination of bacterial antagonists (*Flavobacterium* sp. and *Pseudomonas* sp.) introduced 17 days after transplanting which did not differ significantly with untreated-inoculated plants.

In terms of yield, plants treated with garlic extract combined with *Pseudomonas* sp. and *Flavobacterium* sp. produced good quality cutflowers. The color of the flowers was bright orange compared with the untreated inoculated plants which were small and pale. The treatments produced lower non-marketable comparable to the fungicide treated plants. The plants applied with the combination of hot water treatment + garlic extract + combination of bacterial antagonists introduced 17 days after transplanting (DAT) produced more of class B and non-marketable flowers.

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INTRODUCTION

Background of the Study

Fusarium wilt represents a continuing challenge for worldwide production of chrysanthemum. It causes significant losses in chrysanthemum crops. These losses may occur year after year because of the carry over of the organisms in infected propagation stocks, the persistence of the fungus in the soil and the difficulty in controlling the fungus once it becomes established in the soil (Engelhard and Woltz, 1973). This is caused by the fungus, *Fusarium oxysporum f. sp. chrysanthemi*. The disease is frequently complicated by the variability of symptoms on various cultivars and because the symptoms may resemble those of nutrient deficiencies, *Pythium* root rot or excess water (Engelhard and Woltz, 1971). Infection results in plugging of the xylem vessel elements with gum and pectinaceous materials, hypertrophy and hyperplasia of xylem parenchyma cells, abnormal activity of the vascular cambium derivatives, the formation of cavities within xylem tissues and eventual colonization of phloem and cortex parenchyma cells that results in their collapse (Bowers and Locke, 2000).

Most of the growers are aware of this dreaded disease , however, very few know how to manage the disease properly. When the disease attacks, it usually appears at reproductive stage thus normally affects the quality and quantity of the produce. Farmers apply control measures however, they do it improperly and at the wrong time. Agri-chemicals play an important role in

improving the quality of cutflowers harvested. However, use and misuse can give detrimental effect to the environment and to public health. Chemicals pollute the environment, enter the food chain, harm beneficial or non-target organisms and contaminate the water supply. Moreover, chemical pesticides are ineffective against most soil-borne pathogens and the control action of the effective ones is often short-lived (Madamba et al., 1999; Agrios, 1997).

Importance of the Study

Biologically-based and environmentally-safe alternatives such as the use of biological control agents, natural plant products, and cultural methods are being investigated for possible use in integrated management program (Bowers and Locke, 2000). Biological control along with accurate disease detection, diagnosis, and sound cultural management techniques offer the best alternative measure to reduce pesticide use in chrysanthemum production.

In the highlands of Northern Philippines, the first report on the use of bacterial antagonists and plant extracts for the control of *Fusarium* wilt in garden pea was done by Villanueva and Lirio (2000). *Flavobacterium* sp. isolated from healthy beans at Sagada, Mt. Province provided the best control of the disease and the highest yield. Among the plant extracts, *Psidium guajava* provided the highest percentage control followed by *Piper betle.* The efficacy of garlic extract and bacterial antagonists against *F. oxysporum f. sp. chrysanthemi* has been shown in the previous trials (Villanueva et al , 2004).

Objectives of the Study

This study was therefore conducted with the following objectives:

1. to determine the effect of selected bacterial antagonists, plant extracts, and fungicides against *F. oxysporum* f. sp. *chrysanthemi* under laboratory conditions;

2. to determine the compatibility of selected plant extracts and fungicides with the bacterial antagonists;

3. to determine the effectiveness of the bacterial antagonists and plant extracts in reducing the soil population of *F.oxysporum* f. sp *chrysanthemi*; and

4. to determine the effect of bacterial antagonists and plant extracts on *Fusarium* wilt infection and cutflower yield.

Time and Place of the Study

The experiments were conducted at the Biocon Laboratory and greenhouse of the Horticulture Research and Training Institute (HORTI), Benguet State University, La Trinidad, Benguet from November, 2005 to October, 2006.

REVIEW OF LITERATURE

The Disease

In chrysanthemum, the fungus that caused *Fu*sarium wilt is composed of two races of *Fusarium oxysporum f. sp. chrysanthemi* (Forsberg, 1976), each of which attacks specific chrysanthemum varieties. In addition, disease development depends on the crop or variety, the race of *Fusarium* present and environmental factors such as temperature, nitrogen and soil reaction (Nyvall, 1979; Singh, 1978; Agrios, 1988).

Fusarium wilt is one of the most widespread and destructive diseases of major ornamental and horticultural crops. The soil-borne fungus causes vascular wilts by infecting plants through the roots and growing internally through the cortex to the stele. The vascular tissues of the root and then the stems, are colonized by growth of hyphae and movement of conidia in the transpiration stream. Initial symptoms appear as chlorosis, and distortion of the lower leaves, often on one side of the plant. Foliar chlorosis, necrosis and plant stunting become more pronounced as the disease progresses. Wilting occurs on the affected side of the plant, followed by vascular discoloration and stem necrosis. The entire plant wilts and dies as the pathogen moves into the stem (Bowers and Locke, 2000).

According to Agrios (1997) *F*usarium wilt damages plant by causing stunting until it soon wilts and finally die. These pathogens do not reach the apical meristem until the very late stages of the disease. This is characterized by

drying of the entire plant due to the toxic substances secreted by the pathogen attacking the plant (Forsberg, 1976).

Symptoms

According to Nyvall (1979) the typical symptoms appear as yellowing (chlorosis) and distortion of leaves on one side of the plant. Chlorosis begins on the lower leaves followed by wilting that progresses up the plant. In the early stages of the disease, the roots are not rotted. In many plants such as carnation and gladiolus, the infection may be one sided at first. Infected plants wilt, the lower leaves turn yellow and dry and the xylem tissues turn brown followed by death of the plant (Agrios, 1997 ; Bowers and Locke, 2000).

The occurrence of specific symptoms and their severity depends primarily on the interaction of the cultivar and temperature of the soil and air. The pattern of symptom development in cultivars also varies wherein symptoms appear first at the plant apex and move down in some cultivars, rather than starting at the base of the plant as in most vascular wilts. Temperature above 28ºC favor disease development and play a major role in symptom development (Stuehling *et. al*. 1981).

Sign

The fungus *F. oxysporum* f. sp. *chrysanthemi* has two types of conidia: macroconidia (large, multi-celled spores) and microconidia (small, one-celled

spores) (Bowers and Locke, 2000). According to Agrios (1997) under the microscope, macroconidia appear as crescent-shaped, hyaline, slimy, banana-shaped and septated while microconidia appear as hyaline and single-celled.

Survival

F. oxysporum f. sp. chrysanthemi like other species survived in the soil saprophytically almost forever (Agrios, 1988 and 1997; Roberts and Boothroyd, 1972and Bowers and Lock, 2000). It is generally spread through water splash, irrigation, contaminated tools and contaminated planting materials. Once within the plant, the fungus grows and multiplies in the vascular system (water and food conducting tissues) of the roots. It then moves upward in the plant by spores (macrocinidia and micro conidia) that are transported in the sap stream where they become lodged, germinate and affect new plant parts; or the fungus extends its colonization as it grows in the vascular tissues of the host. The normal flow of liquids and nutrients from the roots to the foliage is greatly reduced or stopped because the conducting tissue becomes partially plugged or killed by fungal mycelium and spores, or by the overgrowth of the neighboring cells. Toxic substances are believed to be secreted by interaction of the fungus and the host plant. These materials apparently cause the wilting and eventual death of the plant. Wilt symptoms typically are not observed until the fungus has colonized the underground parts of the plant.

Resting structures (chlamydospores) are formed within infected plant

parts. After the host plant dies or the growing season ends, this *Fusarium* fungi survive as mycelia and chlamydospores, overwintering in dead plant parts; or may live in the soil indefinitely in the absence of the host plant, especially if the soil is warm, as in the greenhouse. Chlamydospores are stimulated to germinate by exudates from the roots of a host plant which they then infect (Forsberg, 1976; Bowers and Locke, 2000). *Fusarium* wilts are much more common and destructive in the warmer temperate regions and in the tropics and subtropics becoming lesser damaging or rare in colder climates except for greenhouse crops in these areas (Agrios, 1997). According to Forseberg (1976) severe symptoms develop at constant temperatures of 80ºF and 90ºF. They are mild at 70ºF and absent at 60ºF or below. Soil temperatures of about 25ºC (77ºF) and higher and low soil moisture generally favor wilt development (Singh , 1978). On the other hand, (Nyvall , 1979) also reported that disease severity is greater during high temperatures (35ºC day and 29 ºC night), moist weather and in plants grown in acidic and light textured soils that are high in nitrogen. Constant high temperatures are known to favor development of *Fusarium* wilt of chrysanthemum caused by *Fusarium oxysporum f. sp. chrysanthemi.* In a previous study, a constant temperature of 35ºC was most favorable for development of this disease (Gardiner *et. al.* 1989).

In the presence of roots, chlamydospores or conidia germinate and penetrate susceptible plants. The fungus enters the xylem and grows upward

plugging the tissues and reducing the movement of water. Toxins are produced that cause the foliage to turn yellow but an infected plant may remain symptomless at lower temperatures.

Management

Control of *Fusarium* wilt of chrysanthemum involves the use of culture indexed cuttings, treatment of the growing medium and sanitation. Culture-indexing is widely used in the industry to assure that cuttings are free of the pathogens that cause *Fusarium* and *Verticillium* wilts. Sanitation is used to keep the medium free of the pathogen during the growing period (Toop, 1963).

Fungicide drenches and growing potted chrysanthemums in a high lime, all nitrate nitrogen cultural regime have been shown to provide complete management of *Fusarium* wilt. However, plants may still be colonized by the fungus and should not be used as a source of cuttings for propagation Engelhard and Woltz (1971).

Biological Control

Biological control is becoming an urgently needed component in agriculture. Chemical pesticides have been the objects of substantial criticism in recent years due to the adverse environmental effects causing health hazards to human and other non-target organisms including beneficial natural enemies. It is now safer and environmentally feasible control alternatives the use of existing

living organisms to provide protection against a large range of plant pathogenic fungi without damage to the ecological system (Madamba et. al., 1999).

Biological control is the use of any organisms to control a pathogen. It is the reduction in attack of a crop species by a pathogen achieved using another living organisms. This includes both direct and indirect effects due either to introduced antagonist or manipulation of existing populations to reduce disease (Baker and Cook, 1974).

Becker *et. al* (1993) obtained fluorescent p*seudomonad*s strain from wheat roots grown in take-all decline soils and tested for a possible control of the disease in the field and found out several strains were effective in reducing severity and increasing yield of wheat. On the other hand, Arie et al (1987) also obtained strain M-2196 of Pseudomonas gladioli that was effective in reducing the severity of *Fusarium* wilt of bottle gourd when the roots of *Allium* spp. seedlings were dipped in the bacterial suspension before being transplanted along side bottle gourds plants.

In addition, strains of fluorescent P*seudomonads* PGPR that promoted potato yield induced reduction in root populations of the potato soft-rot pathogen, *Erwinia carotovora* of 95-100% and a reduction in the percentage of daughter tubers infected with the pathogen ranging from 28-92% compared to non-treated controls (Bolayo, 1996). PGPR has been reported as potential biological control agents for many root and crown rot pathogens including *Aphanomyces*, *Fusarium*

solani, Gauanomyces graminis var.*tritici, Phytophthora megasperma* f. sp., g*lycinia, Sclerotium rolfsii* and *Thielaviopsis bassicola*. These provided protection by diverse mechanisms including production of antibiotics, production of extra - cellular lytic enzymes, and production of hydrogen cyanide (Bowers and Locke, 2000).

Villanueva and Lirio (2000) reported that out of 160 bacterial isolates collected from vegetable growing areas in Benguet and Mt. Province, two isolates showed strong potential against *Fusarium* wilt pathogen. *Flavobacterium* sp. obtained from healthy beans at Sagada, Mt. Province provided the best control (53.76%) followed by the standard fungicide, mancozeb and *Bacillu*s sp. with 53.3 and 48.10 % control, respectively. Likewise, the highest pod yield was obtained from plants applied with *Flavobacterium* sp followed closely with plants treated with *Bacillus* sp.

Plant Extracts

Antimicrobial activity of plant extract is attributed to the presence of bioactive compound (s) as reported by some researchers (Favaron et. al., 1993: Onu, 1995; Rao and Singh, (1992) and Villanueva and Lirio, (2000). Aqueous extract of *Piper betle* was reported to demonstrate antifungal activity against several test fungi. The active volatile principle was identified as eugenol. There was in fact, the first report of the antimicrobial activity of *P. betle* ethanol extract at concentration of 1.0 g per liter and 2.5 g per liter when tested both in - vitro and

in - vivo respectively against three fungal pathogens namely, *Pyricularia oryzae*, *Cochliobolus miyabeanus* Deschler and *Rhizoctonia solani* Kuhn which incite blast, brown spot and sheath blight diseases of rice, respectively (Villanueva and Lirio, 2000).

The high levels of polyphenols from *P. betle* leaves (chavicol, chavibetol, allylpyro catechol, chabetol acetate and allylpyrocatecol diacetate) were considered responsible for this fungicidal and nematicidal activities (Villanueva and Lirio (2000).

According to Saxena (1983) plants are virtually "nature's chemical factories" providing practically unlimited natural resources of botanical pesticides. Plant themselves produced highly sophisticated defense chemicals which contain abundance of natural defense systems. Different societies in the world have continually employed plants to kill or repel pests since civilization began. However, due to appearance of synthetic chemicals, this kind of indigenous technology was ignored (Wagang, 1999).

According to Villanueva and Lirio (2000) *Psidium guajava* provided the highest percentage control (44.81%) of *Fusarium* wilt in garden pea. This was not, however, significantly different from the degree of control exhibited by *P. betle* (21.68%). Greenhouse experiment conducted by Villanueva and Masangcay (2004) showed that 10% garlic extract significantly reduced the soil population density of *F. oxysporum* f. sp. *chrysanthemi.*

Chloride and Soil pH.

Chloride nutrition has proven useful in the suppression of *Fusarium* diseases in many crops that have tolerance to Cl. A single application of 0.25 – 0.5g of Na CL/L soil applied to cyclamen plugs grown in soil infested with *Fusarium oxysporum* decreased mortality and increased fresh weight and leaf area. The most noticeable effect was its ability to postpone the onset of wilt symptoms and delay disease severity. Plant tissue analysis revealed elevated levels of Na, Cl, and Mn. Since Mn is associated with the defense mechanism in plant tissue, this may be one mechanism by which NaCl suppresses F*usarium* wilt. Adding lime to a potting mix to raise to pH from 6.5 to 7.5 did not result in any significant effect on the disease, plant weight or flower number. Also, no significant differences in growth or disease were noted when the pH of medium was lowered to 5.5 with sulfuric acid. When NaCl was combined with the different pH treatments, NaCl improved growth, but the greatest benefit was seen at a pH of 7.5 pH. This may be due to the NaCl increasing Mn and other trace elements at the higher pH (Elmer, 2000).

MATERIALS AND METHODS

Collection and Isolation of the Pathogen

Plants infected with Fusarium wilt were collected from greenhouses where chrysanthemum plants are grown. The diseased specimens were brought to the biocontrol laboratory for isolation and washed thoroughly. From the advancing lesion, two sections were cut and disinfected with 1% chlorox for three minutes then rinsed three times in sterile distilled water. The lesions were blotted dry in sterilized tissue paper. After which, four sections of the cut specimen were transferred equidistantly in previously plated potato dextrose agar (PDA) and then placed in the incubation chamber with an average temperature of 27ºC for 5 days.

Laboratory Experiment

Bioassay Test

The effect of bacterial antagonists, plant extracts and selected fungicides on the growth of *F. oxysporum* f. sp. *chrysanthemi* was determined.

In preparing inoculum suspension, the plates containing pure culture of *F. oxysporum* f. sp. *chrysanthemi* were dispensed with 10 ml sterile distilled water (SDW). Using sterilized wire loop, the fungal growth was scraped and the inoculum suspension was pre-standardized by counting the number of spores per ml using a haemacytometer. A fungal suspension with spore concentration of 1.2×10^6 was used in the bioassay test. On sterilized petri plate, 0.1 ml of the

fungal suspension was placed and evenly distributed into each sterilized plate. Later, PDA medium was then poured and spread uniformly in each plate and incubated for 5 days.

Preparation of bacterial suspension

Pure cultures of the bacterial antagonists maintained in the Biocon Laboratory were sub-cultured by re-streaking into solidified agar slants containing nutrient agar medium. After incubation at 28ºC for 24 hours, the bacterial cells were suspended in SDW and standardized to contain ca. 1 x 10^7 cfu/ml. Nine sterile paper discs were dipped into the suspension of the antagonistic microorganisms. Three discs were equidistantly placed at the center of the plate. The treatments were replicated three times and arranged randomly in the improvised incubation chamber following the completely randomized design (CRD). The diameter of inhibition zone was measured for each replication after 24 hours of incubation.

Preparation of Plant Extracts

Leaves of gawed (*Piper betle*) and kutsai (*Allium schoenoprasum* L.) , bulbs of garlic (*Allium sativum* L) and red onions (*Allium cepa*) and hot pepper fruits (*Capsicum frutescens* L.) were brought to the laboratory and washed thoroughly. Twenty grams of the materials were added to 20 ml of sterilized distilled water. Using mortar and pestle, the materials were macerated and the

sap was extracted using sterilized cheese cloth (Plate 1).

Plate1. Extraction of plant extracts for bioassay tests.

Preparation of Fungicides

To prepare the fungicide solution to be used in the bioassay test, the computation was based on recommended rates. The desired amount was weighed using an electronic balance. Sterile filter paper discs were soaked in each chemical solution and placed at the center of the solidified PDA medium prepared earlier. The treatments were replicated three times and arranged randomly in the improvised incubation chamber. The diameter of inhibition zone was measured after three days.

The different treatments were:

TREATMENTS	ISOLATES/ LOCAL NAME	SCIENTIFIC NAME/ CHEMICAL NAME	CONCENTRATIONS/ RECOMMENDED RATE
T1	Control		
T2	Isolate 31	*Bacillus sp.*	10^7 cfu/ml
T3	Isolate 131	*Bacillus pumilus*	10^7 cfu/ml
T4	Isolate 158	*Pseudomonas* sp.	10^7 cfu/ml
T5	Isolate 94	*Flavobacterium* sp.	10^7 cfu/ml
T6	Isolate 73	*Bacillus pumilus*	10^7 cfu/ml
T7		*Verticillium* sp.	10^7 spores/ml
T8	Garlic	*Allium sativum L.*	50%
T9	Gawed	*Piper betle*	50%
T10	Kutsai	*Allium schoenoprasum* L.	50%
T11	Hot pepper	*Capsicum frutescens* L.	50%
T12	Red onion	*Allium cepa*	50%
T13	Fungitox	Thiophanate methyl	0.03g/20ml
T14	Topsin-M	Thiophanate methyl	0.03g/20ml
T15	Parafungus	Mancozeb	0.09 g/20ml
T16	BLB stopper	Thiodazole copper	0.06ml/20ml
T17	Captan	Captan	0.1g/20ml
T18	Benlate	Benomyl	0.03g/20ml
T19	Daconil	Chlorothalonil	0.06g/20ml
T20	Dithane M- 45	Mancozeb	0.08g/20ml
T21	Salt	Sodium chloride	.5g/li H_20

Compatibility Test

The compatibility of the plant extracts and selected fungicides with the bacterial antagonists was tested. This was necessary to determine if plant extracts and fungicides could be integrated to effectively control fusarium wilt of chrysanthemum.

The different treatments are:

T1 *Flavobacterium* sp. + SDW

T2 *Flavobacterium* sp. + Mancozeb (Parafungus)

T3 *Flavobacterium* sp. + Thiophanate Methyl (Fungitox)

T4 *Flavobacterium* sp. + Garlic extract *(Allium sativum* L.)

T5 *Flavobacterium* sp. + Gawed extract (*Piper betle)*

T6 *Flavobacterium* sp. + Table salt(NaCl = .5g/L H_20)

T7 *Pseudomonas* sp. + SDW

T8 *Pseudomonas* sp. + Mancozeb (Parafungus)

T9 *Pseudomonas* sp. + Thiophanate Methyl (Fungitox)

T10 *Pseudomonas* sp. + Garlic extract (*Allium sativum* L.)

T11 *Pseudomonas* sp. + Gawed extract (*Piper betle)*

T12 *Pseudomonas* sp + Table salt (NaCl = .5g /L H_20)

T13 *Pseudomonas* sp. + *Flavobacterium* sp.

Greenhouse Experiments

Effect of bacterial antagonists and plant extracts on the soil population of *Fusarium oxysporum* f.sp. *chrysanthemi*

Two hundred g heat-sterilized soil was placed in each cup and inoculated with *F. oxysporum* f. sp. *chrysamthemi.* Later, the different treatments were applied (Plate 2). Soil samples were taken at 0 (before soil treatment), 1,

3, 7, 14, 21 and 28 days after soil treatment (Plate 3). The population densities of the pathogen were determined using dilution plate techniques (Plate 4).

Plate 2. Application of different treatments.

Plate 3. Collection of soil samples (10g/ replicate)

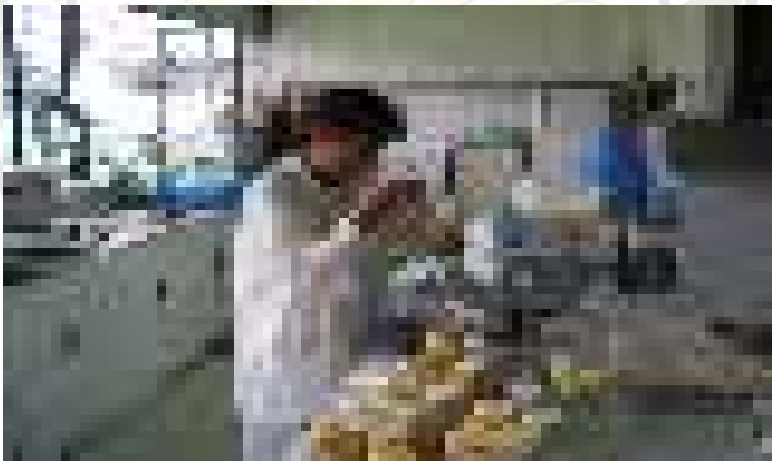


Plate 4. Determination of pathogen population using soil dilution technique

The treatments were:

T1	Uninoculated-Untreated
T2	Uninoculated – Treated
T3	F.o.c. + Hot water treatment
T4	F.o.c. + Hot water treatment + garlic extract after a week + *Flavobacterium* sp. + *Pseudomonas* sp. after 17 DAT
T5	F.o.c. + *Flavobacterium* sp.
T6	F.o.c. + *Pseudomonas* sp.
T7	F.o.c. + *Pseudomonas* sp. + *Flavobacterium* sp.
T8	F.o.c. + Mancozeb (Parafungus)
T9	F.o.c. + Garlic extract (*Allium sativum* L)
T10	F.o.c + Gawed extract (*Piper betle*)
T11	F.o.c + Table salt (NaCl = .5g/Li)

Effect of bacterial antagonists and plant extracts on *Fusarium* wilt infection and chrysanthemum yield

The soil used in the experiment was obtained from an area not previously planted with chrysanthemum. This was brought to the greenhouse for sterilization using a pressure cooker at 15 psi for 2 hours to ensure that all microorganisms in the soil were killed. About two kg soil was placed in 10 cm-diameter plastic pots.

The procedure used for preparing the fungal inoculum, bacterial antagonists and plant extracts was followed. The different treatments were as follows:

T1 Uninoculated/Untreated

T2 Uninoculated/Treated

T3 F.o.c. + Hot water treatment + garlic extract after a week + *Pseudomonas* sp. + *Flavobacterium* sp. after 17 days

T4 F.o.c. + *Pseudomonas* sp. + *Flavobacterium* sp.

T5 F.o.c + Mancozeb (Parafungus)

T6 F. o.c. + Thiophanate Methyl (Fungitox)

T7 F. o.c. + Garlic extract (*Allium sativum* L)

T8 F. o. C. + Gawed extract (*Piper betle*)

T9 F. o.c. + Table salt (1 g/L H_20)

Seedlings of chrysanthemum cv. Toledo were transplanted and arranged randomly in the greenhouse using the randomized complete block design. The treatments were replicated four times with three sample plants per replicate. All cultural management practices for chrysanthemum production were employed uniformly in each treatment such as lighting three hours at night, fertigation, pinching, disbudding (Plate 5), weeding and control of insect pests and foliar diseases. The disease severity was assessed weekly using the modified rating scale by Gardiner (1987) as follows:

SCALE	DESCRIPTION
1	Normal or no infection
2	5-10% leaf foliage showed symptoms of chlorosis or infection
3	11-25% leaf foliage showed symptoms of chlorosis or infection
4	26-50% leaf foliage showed symptoms of chlorosis and show slight symptom of wilting
5	51-75% leaf foliage showed symptoms of chlorosis and show moderate symptom of wilting and a slight vascular rotting
6	76-100% most of the leaf foliage showed symptom of chlorosis and plants are wilting and visual vascular stem rotting

Plate 5. Disbudding of plants

Data Gathered

1. Diameter of inhibition zone for bioassay and compatibility tests. This is the area where pathogen growth is inhibited and was measured in mm using a foot rule to indicate the degree of fungitoxicity of the selected bacterial antagonists, plant extracts and fungicides.

2. Colony forming units (CFU/ml). This was obtained by counting the total number of fungal colonies per plate and computed using the following formula:

$$\text{Cfu} = \frac{\text{Average plate count x dilution factor } (10^4)}{\text{Volume plated (0.1 ml)}}$$

3. Disease severity rating. This was be assessed using the modified rating scale of Gardiner (1989).

4. Marketable and non-marketable yield. This was determined by counting the cutflowers produced without damage and non-marketable using the classification of King Louis Flowers and Plants, Inc. for spray type cultivars as follows:

CLASSIFICATION	STEM LENGTH	FLOWER DESCRIPTION
Class AA	> 71	Large flower(s), clean leaves with straight stem
Long	65 – 71	Large medium flower(s), clean leaves with Straight stem
Medium	55 – 61	Medium – small flower(s), clean leaves with straight stem
Short	51	Small flower, clean leaves with straight stem
Class B	41	Small flower(s) with leaf disease
Non marketable		Damaged plants (wilted and diseased)

RESULTS AND DISCUSSION

Laboratory Experiment

Effect of bacterial antagonists, plant extracts and fungicides on the growth of the pathogen

Among the bacterial isolates tested, only *Flavobacterium* sp and *Pseudomonas* sp. showed potential in controlling *Fusarium oxysporum* f.sp. *chrysanthemi* with 1.0 and 0.66 mm inhibition zone, respectively. However, this was comparable with sterile distilled water. *Bacillus* sp., *Verticillium* sp and the two strains of *Bacillus pumilus* did not affect the growth of the pathogen (Table 1 and Plate 6).

Among the plant extracts tested, the widest zone of inhibition was obtained from garlic extract with 9.65 mm. This was significantly different from gawed *(Piper betle*), kutsai (*Allium schoenoprasum)* and hot pepper (*Capsicum frutescens* L.) which were comparable with the control. Similarly, red onion extract (*Allium cepa*) did not inhibit the growth of the pathogen. Antimicrobial activity of plant extracts is attributed to the presence of bioactive compound(s) as reported by most researchers (Favaron *et al*., 1993). The efficacy of the garlic extract may be attributed to the constituents of the garlic which has a broad spectrum anti-bacterial and anti-fungal activity. This antibiotic property is due to the presence of allicin, a S- containing compound in the bulb (Rimando and de Guzman, 1986).

Table 1. Effect of bacterial antagonists, plant extracts and selected fungicides on the growth of *Fusarium oxysporum* f. sp. *chrysanthemi* [a].

	A. BIOCONTROL AGENTS	INHIBITION ZONE (MM
T1	SDW	0e
T2	*Bacillus pumilus* (73)	0e
T3	*Bacillus pumilus* (131)	0e
T4	*Bacillus* sp. (31)	0e
T5	*Flavobacterium* sp. (94)	0.66e
T6	*Pseudomonas* sp. (158)	1.0e
T7	*Verticillium* sp.	0e
	B. PLANT EXTRACTS	
T8	Garlic (*Alliumsativum* L)	9.65ab
T9	Gawed (*Piper betle*)	2.11e
T10	Kutsai (*Allium schoenoprasumL.*)	1.25e
T11	Hot Pepper (*Capsicum frutescens)*	1.66e
T12	Red Onion (*Allium cepa*)	0e
	C. FUNGICIDES	
T13	Thiophanate Methyl (Fungitox)	7.55c
T14	Thiophanate Methyl (Topsin – M)	5.01d
T15	Mancozeb (Parafungus)	11.01a
T16	Thiodazole copper (BLB Stopper)	0e
T17	Captan (Captan)	4.91d
T18	Benomyl (Benlate)	8.78bc
T19	Clorothalonil (Daconil)	0e
T20	Mancozeb (Dithane M-45)	0e
	D. OTHERS	
T21	0.5 g Table salt/Liter	0e

CV = 42.48%

[a] Data are means of four replications. Means followed by a common letter are not significantly different at 5% level using DMRT.

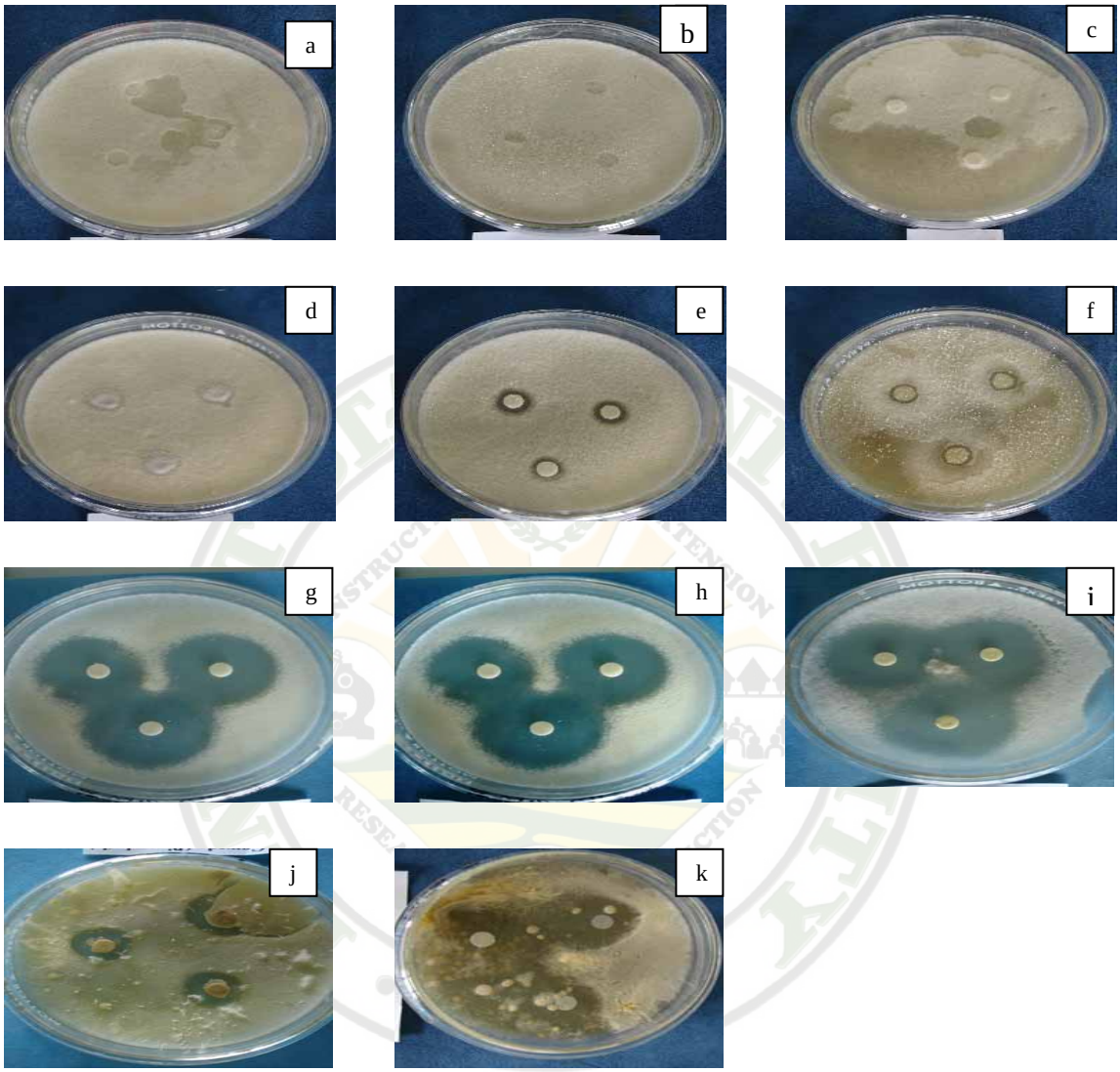


Plate 6. Effect of bacterial antagonists, plant extracts and fungicides on the growth of *F. oxysporum f. sp. chrysanthemi.* *a= Bacillus pumilus; b = Bacillus pumilus ; c = Bacillus sp.; d = Verticillium sp.; e = Pseudomonas sp.; f = Flavobacterium sp.; g* = Garlic extract (*Allium sativum L.)* ; h = Gawed extract (*P. betle);* I = Mancozeb (Parafungus); j = Benomyl (Benlate); and k = Thiophanate Methyl (Fungitox)

On the other hand, aqueous extract of *Piper betle* was reported to demonstrate antifungal activity against several test fungi. The active volatile principle was identified as eugenol (Dubey and Tripathi, 1987). There was in fact a first report of the antimicrobial activity *of P. betle* ethanol extract at concentrations of 1.0 g per liter and 2.5 g per liter when tested both in-vitro and in-vivo, respectively against the three fungal pathogens namely, *Pyricularia oryzae* Cav., *Cochliobolus miyabeanus* Dreschler and *Rhizoctonia solani* Kuhn which incite blast, brown spot and sheath blight of rice, respectively (Tewari and Nayak, 1991).

Among the fungicides tested, the most effective was mancozeb (Parafungus) with inhibition zone of 11.01mm. This was significantly more effective than thiophanate methyl (Fungitox), benomyl (Benlate), thiophanate methyl (Topsin –M) and captan (Captan) with inhibition zones of 7.55, 8.98 5.01 and 4.91 mm, respectively. On the other hand, thiodazole copper (BLB Stopper), chlorothalonil (Daconil) and mancozeb (Dithane M-45) did not affect the growth of the pathogen. This is quite surprising because the active ingredient of dithane M-45 and parafungus are both mancozeb. Table salt applied at 0.5g/liter did not inhibit the growth of *F. oyxsporum* f. sp. *chrysanthemi*.

Compatibility Test

The effect of plant extracts and selected fungicides using their recommended rate on the growth of the bacterial antagonists is shown in Fig. 1 and Plate 7. Garlic extract and mancozeb significantly inhibited the growth of *Flavobacterium* sp. and *Pseudomonas* sp. On the other hand, thiophanate methyl, gawed extract and table salt did not affect the growth of the above bacterial antagonists. This implies that plant extracts and fungicides are compatible with the biocontrol agents and could therefore be used in a sustainable management of *Fusarium* wilt of chrysanthemum.

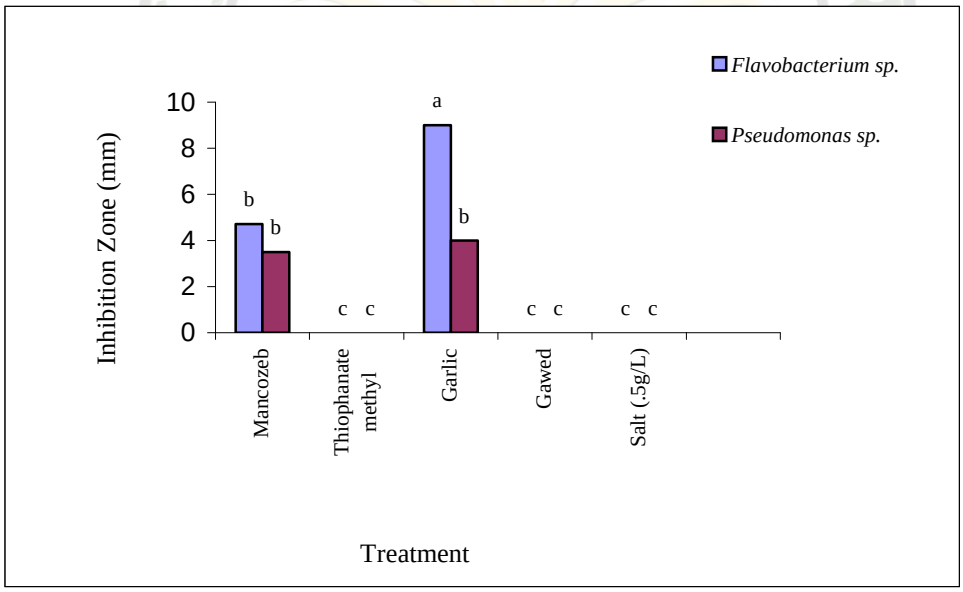
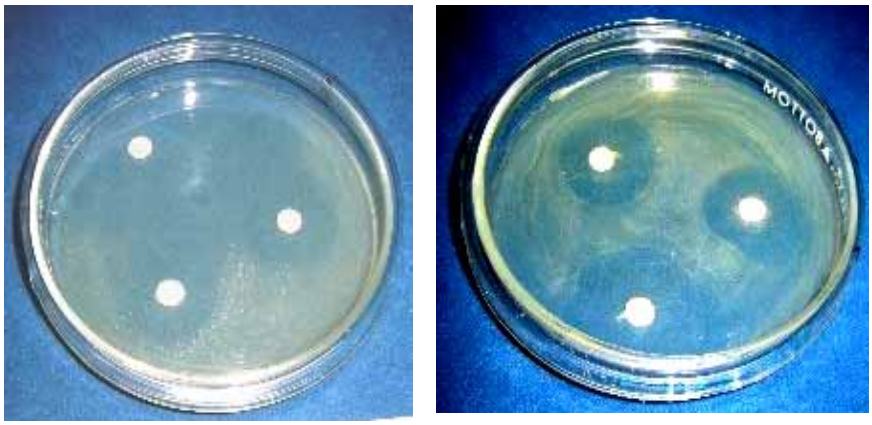
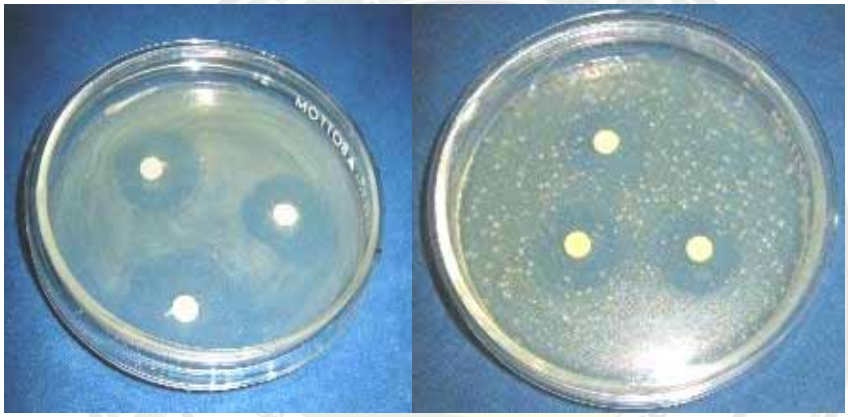


Fig 1. Effect of plant extracts and selected fungicides on the growth of bacterial antagonists. Bars with a common letter are not significantly different at 5% level using DMRT.



Flavobacterium sp. + Garlic extract

Flavobacterium ***sp.*** + Mancozeb (Parafungus)



Pseudomonas sp. + Mancozeb (Parafungus)

Pseudomonas sp. + Garlic extract

Plate No.7 . Effect of garlic extract (*A. sativum* L.) and mancozeb (Parafungus) on the growth of bacterial antagonists: *Pseudomonas* sp. and *Flavobacterium* sp.

Greenhouse Experiment

Effect of Bacterial Antagonists and Plant Extracts on the Soil Population of *F.oxysporum* f. sp. *chrysanthemi*

Fig. 2 shows the effect of the different treatments on the soil population of *F. oxysporum* f.sp. *chrysanthemi*. Apparently, soil treated with hot water, *Flavobacterium* sp., combination of *Flavobacterium* sp. and *Pseudomonas* sp. and the standard fungicide, mancozeb significantly reduced the population density of the pathogen a day after application. Three days after soil treatment, a decrease in population was noted in all the treatments except those applied with gawed extract and table salt. However, aside from mancozeb and the combination of the two bacterial antagonists, *Flavobacterium* sp. and *Pseudomonas sp., a*ll the treatments reduced their efficacy after a week. This implies that the efficacy of the treatments was only good for three to seven days. One possible explanation is that most of the pathogens died after treatment but the surviving populations multiplied rapidly resulting to the increase of the population on the seventh day after treatment. At this time the pathogen had reached its maximum growth so competition for food and space occurred leading to the death of the organisms. On the 14th day, the population started to decline due to lack of food. Application of garlic extract a week after hot water treatment resulted in significant reduction in soil population of *F. oxysporum* f. sp. *chrysanthemi*. This is reflected in the data 14 days after treatment.

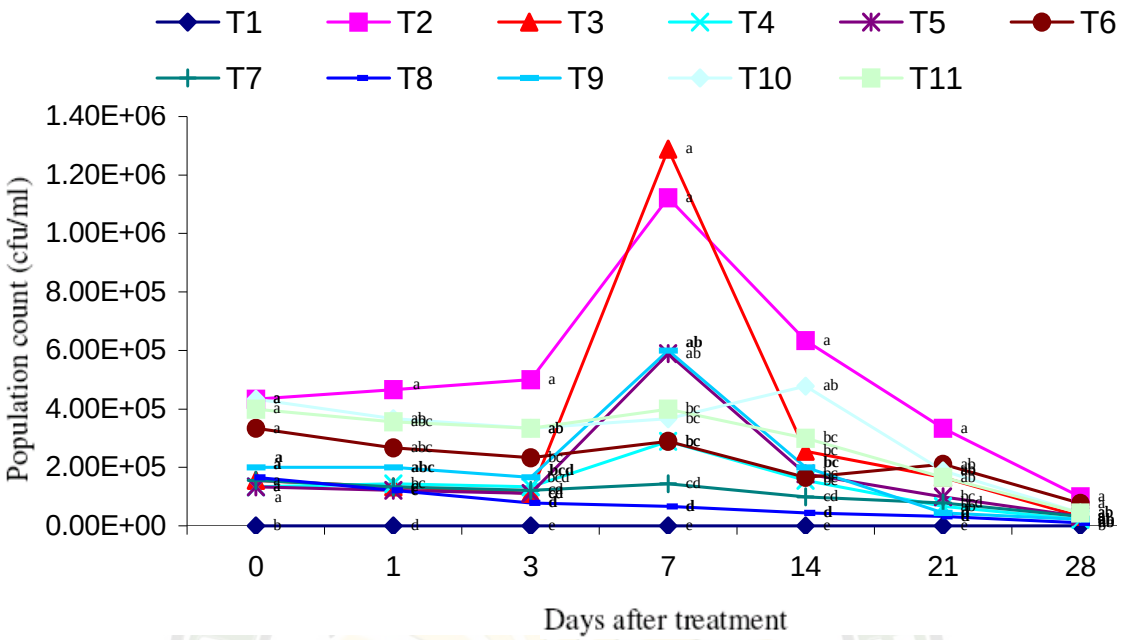


Fig. 2. Effect of bacterial antagonists and plant extracts on the soil population of *F. oxysporum* f. sp. *chrysanthemi*. Lines with a common letter are not significantly different at 5 % level using DMRT. Note: 1- Uninoculated/untreated, 2-untreated/inoculated, 3-F.o.c. + Hot H2O Treatment . 4- F.o.c. + Hot H_2O + after 1week add garlic extract + Comb. Of *Pseudomonas* sp. + *Flavobacterium sp 17DAT.*, 5- F.o.c. + *Flavobacterium sp.*, 6- *F.o.c.* + *Pseudomonas sp.*, 7- - F.o.c. + *Pseudomonas sp.* + *Flavobacterium sp.*, 8- Parafungus (Mancozeb), 9- Garlic extract (*Allium sativum* L.), 10- Gawed extract *(Piper betle*), 11- Table Salt (.5g/L).

Except for *P. betle* which was comparable with the untreated control, all the other treatments gave significantly lower population of the pathogen. Twenty one (21) days after treatment, the population of *F. oxysporum* f. sp. *chrysanthemi* was lower in the soil applied with mancozeb, garlic extract, combination of *Flavobacterium* sp. and *Pseudomonas* sp., the integration of hot

water, garlic extract and bacterial antagonists while those treated with hot water, gawed extract, table salt and *Pseudomonas* sp. alone did not differ from the untreated control. Finally, a reduction in the soil population was noted 14 days after treatment and gradually declined up to the termination of the experiment. During the last assessment period which was taken 28 days after treatment, all the treated soil were comparable with the untreated ones. It can also be noted that a week after treatment, a gradual decline in the population of the pathogen was observed in the untreated soil. Population decline could be due to the absence of host or lack of food.

The observed reduction in the pathogen population in the soil treated with garlic and bacterial antagonists may have an important role in biologically-based management strategies for *Fusarium* wilt. One possible management could be the incorporation into the soil to initially reduce the pathogen population. This would be followed by application of biological control agent compatible with the extract three to ten days later to rapidly colonize the treated soil and to further suppress the development of the pathogen thus achieving sustainable disease control of *Fusarium* wilt of chrysanthemum.

Effect of bacterial antagonists and plant extracts on f*usarium* wilt infection and chrysanthemum yield.

Fusarium wilt infection

Figure 3 shows the effect of bacterial antagonists and plant extracts on *Fusarium* wilt infection. Apparently, the most effective was thiophanate methyl, however, it was not significantly different with that of mancozeb and garlic extract. Although not as effective as the standard fungicides tested, application of *Pseudomonas* sp. and *Flavobacterium s*p. and table salt significantly reduced *Fusarium* wilt infection compared with the untreated inoculated plants. Application of gawed extract and hot water treatment followed by garlic extract and the combination of bacterial antagonists did not suppress the growth of the pathogen. The potential of garlic extract and the two bacterial antagonists for the control of Fusarium wilt in chrysanthemum has been reported by Villanueva *et al.* (2004). The efficacy of the extract is attributed to the constituents of garlic which has a broad spectrum anti-bacterial and anti-fungal activity. This antibiotic property is due to the presence of allicin, an S-containing compound in the bulb (Rimando and de Guzman, 1986). Since the inhibition zone obtained in *Pseudomonas* sp. and *Flavobacterium* sp. was not so wide in the bioassay test, it is presumed that the mode of action of the above antagonists is not antibiosis, but could be any of the following mechanisms: parasitism, competition or rhizosphere

competence. On the other hand, the potential of the table salt for the control of *Fusarium* wilt was also shown in the experiment. According to Elmer (2000) chloride nutrition has proven useful in the suppression of *Fusarium* wilt diseases in many crops. A single application of salt at the rate of 0.25-.5 g/ liter soil applied to cyclamen plugs grown in soil infested with *F. oxysporum* decreased mortality and increased fresh weight and leaf area. The most noticeable effect was the ability of salt to postpone the onset of wilt symptoms and delay disease severity. Plant tissue analysis revealed elevated levels of Na, Cl and Mn. Since Mn is associated with the defense mechanism in plant tissue, this may be one of the mechanisms by which table salt suppresses *Fusarium* wilt. Gawed extract was not effective against *Fusarium* wilt of chrysanthemum. This result is similar to the findings of Villanueva and Lirio (2000) on Fusarium wilt in garden pea. According to Sullivan (2004) direct inoculation of beneficial organisms like *Trichoderma* spp., *Flavobacterium* spp., *Streptomyces sp., Gliocladium* spp., *Bacillus* spp. and *Pseudomonas* spp. could effectively control soil-borne pathogens.

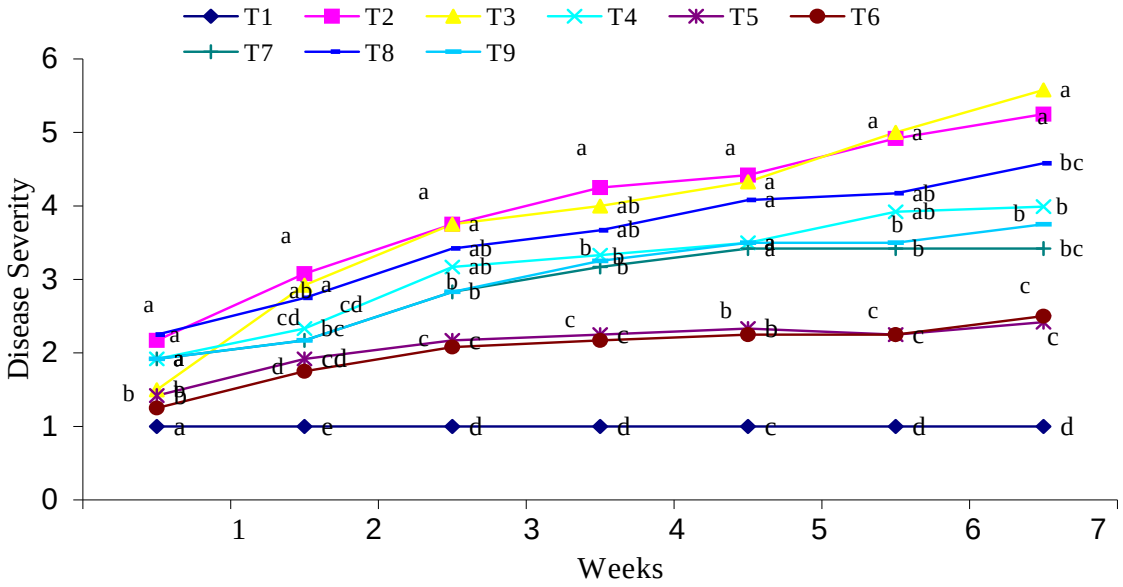


Figure 3. Effect of bacterial antagonists and plant extracts on *Fusarium* wilt infection of chrysanthemum. Points with a common letter are not significantly different at 5 % level using DMRT. Note: 1-Uninoculated/untreated, 2-untreated/inoculated, 3-F.o.c. + Hot H2O + after 1week add garlic extract + comb. of *Pseudomonas* sp. + *Flavobacterium sp 17DAT.*, 4- *Pseudomonas sp.* + *Flavobacterium sp.*, 5- Mancozeb (Parafungus), 6- Thiophanate methyl (Fungitox), 7-Garlic (*A. sativum* L.), 8- Gawed *(P.betle*), 9- Table Salt (1g/L H_20).

Vascular discoloration

Disease severity in terms of length of vascular discoloration was shortest in plants applied with thiophanate methyl. However, the effect was not significantly different from plants applied with mancozeb, combination of *Pseudomonas* sp. and *Flavobacterium* sp., garlic extract, table salt and gawed extract. On the other hand, application of hot water treatment followed by garlic extract and the combination of bacterial antagonists was not effective in

controlling the disease (Table 2 and Plate 8). It is possible that the temperature of hot water was not high enough to completely kill the pathogen. In addition, there is possibility that the slight increase in soil temperature may have favored the growth and multiplication of *F. oxysporum* f. sp. *chrysanthemi*.

Table 2. Effect of bacterial antagonists and plant extracts on the vascular discoloration expressed as disease severity [a]

TREATMENT	DISEASE SEVERITY
Uninoculated/Untreated	0.00 c
Uninoculated/Treated	8.083 a
F.o.c. + Hot H20 + add garlic extract after a week + comb. of *Pseudomonas sp.*+ *Flavobacterium* sp after 17days	8.875 a
Pseudomonas sp. + *Flavobacterium* sp.	2.878 bc
Mancozeb (Parafungus	2.210 bc
Thiophanate methyl (Fungitox	1.335bc
Garlic extract (A. Sativum L.)	2.793 bc
Gawed extract (P. Betle)	4.045 b
Table salt (1 g/L H_20)	3.020 bc
CV	57.96%

[a] Data are means of four replications. Means with a common letter are not significantly different at 1% level using DMRT

Plate 8. Vascular discoloration as affected by bacterial antagonists, plant extracts and fungicides. a=uninoculated –untreated, b=uninoculated-treated, c= hot h_20 treatment + garlic extract (added after 1 week) + combination of *Pseudomonas* sp. + *Flavobacterium* sp (added 17DAT) , d= comb. of *Pseudomonas* sp. + *Flavobacterium* sp., e=mancozeb (parafungus) f= thiophanate methyl (fungitox), g= garlic extract (*Allium sativum* L.), h= gawed extract (*Piper betle)*, *i*= table salt NaCl=1g/L H_20)

Fresh Weight

Plants treated with the combination of bacterial antagonists gave the highest fresh top weight with mean of 44.50 g. However, this did not significantly differ with the top weights of plants treated with thiophanate methyl, mancozeb, garlic extract and table salt with means of 43.59, 42.84,42.50 and 42.88 g, respectively (Fig.4). The lowest fresh top weight was obtained from plants treated with hot water + garlic extract introduced one week after and the combination of the bacterial antagonists with mean of 3.91 g. The same trend was noted in the fresh root weight: the highest was noted on plants treated with bacterial antagonists followed closely with thiophanate methyl, garlic extract, mancozeb, and table salt with respective means of 6.41, 5.84,5.01, 4.89 and 4.66 g. The lowest fresh root weight was again obtained in plants treated with the integration of hot water, garlic extract and bacterial antagonists with mean of 0.89 g. Although significantly much lower than the above treatments, the fresh top and root weights of plants applied with gawed extract was significantly higher than those obtained in the untreated inoculated plants . According to Adams (1990) susceptible cultivars inoculated with *F. oxysporum* f. sp. *chrysanthemi* exhibit severe symptoms ten days after inoculation and often become severely stunted and die.

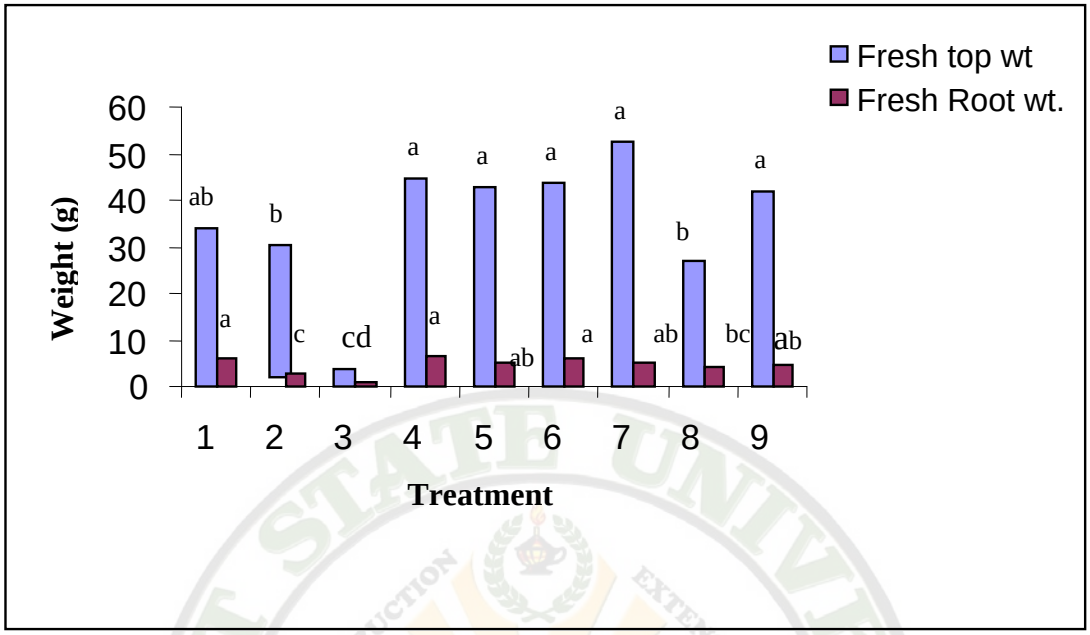


Fig.4. Effect of bacterial antagonists and plant extracts on the fresh weight of plants. Bars with a common letter are not significantly different at 5 % level using DMRT. Note: 1-Uninoculated/untreated, 2-untreated/inoculated, 3-F.o.c. + Hot H2O + garlic extract after 1week + comb. of *Pseudomonas* sp. + *Flavobacterium sp.* after *17days*, 4-*Pseudomonas sp.* + *Flavobacterium sp.*, 5- Mancozeb (Parafungus), 6-Thiophanate methyl (Fungitox), 7- Garlic (*A. sativum* L.), 8- Gawed *(P. betle*), 9- Table Salt (.5g/L).

Cutflower Yield

Plants treated with bacterial antagonists, *Flavobacterium* sp. and *Pseudomonas* sp. produced high quality cutflowers comparable with those treated with standard fungicides, mancozeb and thiophanate methyl (Table 3.) . However, the cutflowers were brighter orange than the latter treatment. In addition, the former treatment produced less non-marketable flowers. On the other

hand, the highest gross sales from cutflower produced was obtained from the untreated uninoculated plants, followed closely by those applied with thiophanate methyl, mancozeb, combination of bacterial antagonists, garlic extract, and table salt with P60.00, P55.83, P 55.00, P48.34 and P40.84, respectively (Table 4). Plants applied with the combination of hot water treatment, garlic extract and bacterial antagonists were not able to produce marketable cutflowers.

Table 3. Effect of bacterial antagonists and plant extracts on the quality of cutflower yield[a]

TREATMENT	CLASSIFICATION				
	LONG	MEDIUM	SHORT	CB	NON MARKETABLE
Uninoculated/ Untreated	0.00b	0.25bc	0.50ab	0.00b	0.00C
Uninoculated/Treated	0.00b	0.25c	0.50b	0.00b	2.25ab
F.o.c. + Hot H20 + garlic extract + comb. Of *Pseudomonas sp.*+ *Flavobacterium* sp.	0.00b	0.00c	0.00b	0.00b	3.00a
Comb. Of P*seudomonas sp.* + *Flavobacterium sp.*	0.50ab	1.00b	0.50ab	1.00 a	0.00c
Mancozeb (Parafungus)	1.00a	0.50bc	1.00a	0.50ab	0.00c
Thiophanate methyl (Fungitox)	1.00a	0.75bc	0.75ab	0.50ab	0.00c
Garlic extract (*A. sativum* L.)	0.25ab	1.00b	0.75ab	0.50ab	0.00c
Gawed extract (*P. betle*)	0.00b	0.75bc	0.75ab	0.00b	1.50b
Table salt (1 g/L H_20)	0.25ab	1.00b	0.75ab	0.00b	1.00b
CV	168.32%	56.53%	103.92%	63.94%	

[a] Data are means of four replications. Means followed by a common letter are not significantly different at 5% level using DMRT

Table 4. Effect of bacterial antagonists and plant extracts on the gross sales of cutflowers.

TREATMENT	CLASSIFICATION / GROSS SALE (PhP)					
	LONG	MEDIUM	SHORT	CB	NON-MARKETABLE	TOTAL
Uninoculated/Untreated	0.00	60.00	0.00	0.0	0.00	P60.00
Uninoculated /Treated	0.00	5.00	8.34	0.00	0.00	P13.84
Hot H_20 treatment + garlic extract (1 week after) + comb. of *Pseudomonas* ap. & *Flavobacterium* sp after 17 days	0.00	0.00	0.00	0.00	0.00	0.00
Comb. o *Pseudomonas* sp. & *Flavobacterium* sp.	11.66	20.00	8.34	10.00	0.00	50.00
Mancozeb (Parafungus)	23.32	10.00	16.68	5.00	0.00	55.00
Thiophanate methyl (Fungitox)	23.32	15.00	12.51	5.00	0.00	55.83
Garlic extract (*A. sativum* L)	5.83	20.00	12.51	10.00	0.00	48.34
Gawed extract (*P. betle*)	0.00	15.00	12.51	10.00	0.00	37.51
Table Salt (.5g/L H_20)	5.83	20.00	12.51	2.50	0.00	40.84

SUMMARY, CONCLUSION AND RECOMMENDATION

SUMMARY

The study aimed to; 1. determine the effect of selected bacterial antagonists, plant extracts and fungicides on the growth of *Fusarium oxysporum* f. sp. *chrysanthemi;* 2. determine the compatibility of plant extracts and fungicides with bacterial antagonists, 3. determine the effectiveness of bacterial antagonists and plant extracts in reducing the soil population of *F. oxysporum* f. sp. *chrysanthemi* and 4. determine the effect of bacterial antagonists and plant extracts on *Fusarium* wilt infection and yield of chrysanthemum.

Results of the in-vitro bioassay test showed that the growth of *F. oxysporum* f. sp. *chrysanthemi* was not significantly inhibited by bacterial antagonists *Flavobacterium* sp. and *Pseudomonas* sp. Garlic extract (*Allium sativum* L) gave the widest inhibition zone comparable to the standard fungicides, mancozeb (Parafungus) and thiophanate methyl (Fungitox).

Mancozeb and garlic extract are not compatible with the bacterial antagonists while thiophanate methyl and gawed extract did not affect the growth of *Flavobacterium* sp. and *Pseudomonas* sp. Combination of *Flavobacterium* sp. and *Pseudomonas* sp. was comparable with the standard fungicide mancozeb. Although not as effective as the other treatments, application of garlic extract, *Flavobacterium* sp. and *Pseudomonas* sp. alone and table salt significantly reduced the soil population of the pathogen compared to the untreated inoculated

plants. Gawed extract was inferior with the above treatments in suppressing the pathogen population.

In the greenhouse trial, the lowest fusarium wilt infection was obtained from plants applied with mancozeb, thiophanate methyl and garlic extract. Although not as superior as the other treatments, the combination of *Pseudomonas* sp and *Flavobacterium* sp. and application of table salt significantly reduced the disease infection in chrysanthemum. On the other hand, integration of hot water treatment, garlic extract and the combination *Pseudomonas* sp. and *Flavobacterium* sp. did not give promising results.

Plants applied with thiophanate methyl and mancozeb gave the best quality cutflowers comparable with the uninoculated control. However, did not significantly differ with plants applied with *Pseudomonas* sp. + *Flavobacterium* sp. Application of the bacterial antagonists resulted in more bright and intense colored flowers which added to the overall quality of the cutflowers. The poorest quality cutflowers were obtained from plants treated with hot water + garlic extract + *Pseudomonas* sp. and *Flavobacterium* sp. and the untreated-uninoculated plants.

CONCLUSION

Combination of bacterial antagonists, *Pseudomonas* sp. & *Flavobacterium* sp. and garlic extract could be potential alternatives to soil fungicides in the management of *F. oxysporum* f. sp. *chrysanthemi* in chrysanthemum.

RECOMMENDATION

1) Combination *of Pseudomonas sp. and* Flavobacterium sp. and garlic extract are recommended for managing fusarium wilt under greenhouse conditions. However, another trial is necessary , preferably in the open field to verify the efficacy of the above treatments.

2) For more effective control, the bacterial antagonists should be applied weekly to give sustainable control of the disease; and

3) For more convenient application, a more rapid and easy technique of extracting garlic juice should be studied.

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APPENDICES

APPENDIX TABLE 1. Inhibition zone (mm) produced by biocontrol agents, plant extracts and fungicides against *Fusarium oxysporum f. sp. chrysanthemi* after 5 days (actual)

TREATMENT	REPLICATION I	II	III	Total	Mean
A. Biocontrol Agents					
Sterile Distilled Water (SDW)	0.0	0.0	0.0	0.0	0.0
Isolate 31 (*Bacillus* sp.)	0.0	0.0	0.0	0.0	0.0
Isolate 131(*Bacillus pumilus)*	0.0	0.0	0.0	0.0	0.0
Isolate 73 (*Bacillus pumilus*)	0.0	0.0	0.0	0.0	0.0
Isolate 94 (*Flavobacterium* sp.*)*	1.0	0.5	0.5	2.0	0.66
Isolate 158 (*Pseudomonas* sp.*)*	1.0	1.0	1.0	3.0	1.0
Verticillium sp.	0.0	0.0	0.0	0.0	0.0
B. Plant Extracts					
Garlic (*Allium sativum* L.)	11.3	9.2	8.43	28.96	9.65
Gawed (*Piper betle*)	4.0	0.0	2.35	6.35	2.11
Kutsai (*Allium schoenoprasum* L)	2.5	1.25	0. 0	3.75	1.25
Hot Pepper (*Capsicum frutescens*)	3.0	0.0	2 .0	5.0	1.66
Red Onion (*Allium cepa*)	0.0	0.0	0.0	0.0	0.0
C. Fungicides					
Thiophanate Methyl (Fungitox)	8.25	7.91	6.5	22.66	7.55
Thiophanate Methyl (Topsin–M)	7.33	4.66	3.05	15.04	5.01
Mancozeb (Parafungus)	13.08	9.91	10.05	33.04	11.01
Thiodazole copper (BLB Stopper)	0.0	0.0	0.0	0.0	0.0
Captan (Captan)	0.0	5.0	3.75	14.75	4.91
Benomyl (Benlate)	11.16	7.16	8.03	26.35	8.78
Chlorothalonil (Daconil)	0.0	0.0	0.0	0.0	0.0
Mancozeb (Dithane M-45)	0.0	0.0	0.0	0.0	0.0
D. Others					
Table Salt (NaCl= .5g /L$H_2$0)	0.0	0.0	0.0	0.0	0.0
GRAND TOTAL				160.9	
GRAND MEAN					2.44

APPENDIX TABLE 2. Inhibition zone (mm) produced by biocontrol agents, plant extracts and fungicides against Fusarium after 5 days (transformed)

TREATMENT	REPLICATION I	II	III	Total	Mean
A. Biocontrol Agents					
SDW	0.71	0.71	0.71	2.13	0.71
Isolate 31 (*Bacillus sp.*)	0.71	0.71	0.71	2.13	0.71
Isolate 131 (*Bacillus pumilus)*	0.71	0.71	0.71	2.13	0.71
Isolate 73 (*Bacillus pumilus*.)	0.71	0.71	0.71	2.13	0.71
Isolate 94 (*Flavobacterium* sp.*)*	1.22	1.00	1.00	3.22	1.07
Isolate 158 (*Pseudomonas* sp.*)*	1.22	1.22	1.00	3.44	1.15
Verticillium	0.71	0.71	0.71	2.13	0.71
B. Plant Extracts					
Garlic (*Allium sativum* L.)	3.44	3.11	2.99	9.54	3.18
Gawed (*Piper betle*)	2.12	0.71	1.69	4.52	1.51
Kutsai (*Allium schoenoprasum L.*)	1.73	1.32	0.71	3.76	1.25
Hot Pepper(*Capsicum frutescens)*	1.87	0.71	1.58	4.16	1.39
Red Onion (*Allium cepa*)	0.71	0.71	0.71	2.13	0.71
C. Fungicides					
Thiophanate Methyl (Fungitox)	2.55	2.35	2.06	6.96	2.32
Thiophanate Methyl(Topsin–M)	2.80	2.27	3.05	5.32	1.77
Mancozeb (Parafungus)	3.69	3.23	3.25	10.17	3.39
Thiodazole Copper(BLB Stopper)	0.71	0.71	0.71	2.13	0.71
Captan (Captan)	2.96	2.90	2.65	5.61	1.87
Benomyl (Benlate)	3.41	2.77	2.92	9.10	3.03
Chlorothalonil (Daconil)	0.71	0.71	0.71	2.13	0.71
Mancozeb (Dithane M-45)	0.71	0.71	0.71	2.13	0.71
D. Others					
Table Salt (.5g/L H_20)	0.71	0.71	0.71	2.13	0.71

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Square	Mean of Squares	Computed F	Prob.
Model	20	813.82	40.69	34.57	0.0001
Treatments	20	813.82	40.69	34.57	0.0001
Error	42	49.43	1.18		
Total	62	863.25			

* - Significant at 5% level using DMRT CV = 42.48%

APPENDIX TABLE 3. Inhibition zone (mm) produced by plant extracts and fungicides against bacterial antagonists; *Pseudomonas* sp. and *Flavobacterium* sp. after 24 hours (actual)

TREATMENT	REPLICATION I	II	III	Total	Mean
Flavobacterium sp. + SDW	0.0	0.0	0.0	0.0	0.0
Flavobacterium sp. + Mancozeb (Parafungus)	5.17	5.0	4.0	14.17	4.72
Flavobacterium sp. + Thiophanate Methyl (Fungitox)	0.0	0.0	0.0	0.0	0.0
Flavobacterium sp. + Garlic (*Allium sativum* L.)	11.75	6.5	8.75	27	9.0
Flavobacterium sp. + Gawed (*Piper betle*)	0.0	0.0	0.0	0.0	0.0
Flavobacterium sp. + .5 g Table salt/li H_20	0.0	0.0	0.0	0.0	0.0
Pseudomonas sp. + SDW	0.0	0.0	0.0	0.0	0.0
Pseudomonas sp + Mancozeb (Parafungus)	4.5	1.0	5.0	10.5	3.5
Pseudomonas sp. + Thiophanate Methyl (Fungitox)	0.0	0.0	0.0	0.0	0.0
Pseudomonas sp. + Garlic (*Allium sativum* L.)	4.25	3.0	4.75	12.0	4.0
Pseudomonas sp. + Gawed (*Piper betle*)	0.0	0.0	0.0	0.0	0.0
Pseudomonas sp. + Table salt (.5g/L H_20)	0.0	0.0	0.0	0.0	0.0
GRAND TOTAL				63.67	
GRAND MEAN					1.93

APPENDIX TABLE 4. Inhibition zone (mm) produced by plant extracts and fungicides against bacterial antagonists; *Pseudomonas* sp. and *Flavobacterium* sp. after 24 hours (transformed)

TREATMENT	REPLICATION I	II	III	Total	Mean
Flavobacterium sp. + SDW	0.71	0.71	0.71	2.13	0.71
Flavobacterium sp. + Mancozeb (Parafungus)	2.83	2.34	2.12	7.29	2.43
Flavobacterium sp. + Thiophanate Methyl (Fungitox)	0.71	0.71	0.71	2.13	0.71
Flavobacterium sp. + Garlic (*Allium sativum* L.)	3.50	2.64	3.04	9.18	3.06
Flavobacterium sp. + Gawed (*Piper betle*)	0.71	0.71	0.71	2.13	0.71
Flavobacterium sp. + .5 g Table salt/li H_20	0.71	0.71	0.71	2.13	0.71
Pseudomonas sp. + SDW	0.71	0.71	0.71	2.13	0.71
Pseudomonas sp + Mancozeb (Parafungus)	2.24	1.22	2.35	5.81	1.94
Pseudomonas sp. + Thiophanate Methyl (Fungitox)	0.71	0.71	0.71	2.13	0.71
Pseudomonas sp. + Garlic (*Allium sativum* L.)	1.18	1.87	2.29	5.34	1.78
Pseudomonas sp. + Gawed (*Piper betle*)	0.71	0.71	0.71	2.13	0.71
Pseudomonas sp. + .5 g Table salt/li H_20	0.71	0.71	0.71	2.13	0.71
GRAND TOTAL				63.67	
GRAND MEAN					1.93

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Square	Mean of Squares	Computed F	Prob.
Treatment	12	290.73	24.23	24.42	0.0001
Error	26	25.80	0.99		
Total	38	316.53			

** - highly significant at 1% CV = 61.01%

APPENDIX TABLE 5. Effect of bacterial antagonists and plant extracts on soil Population count of *F. oxysporum* f. sp. *chrysanthemi* (0 day (before treatment) (actual)

TREATMENT	REPLICATION I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Untreated	200000	600000	500000	1300000	433333.3
F.o.c. + Hot H_20	67000	300000	100000	467000	155666.7
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp + *Flavobacterium* sp.	200000	100000	100000	400000	133333.3
F. o.c + *Flavobacterium* sp.	100000	100000	200000	400000	133333.3
F.o.c + *Pseudomonas* sp.	600000	100000	300000	1000000	333333.3
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	200000	67000	200000	467000	155666.7
Mancozeb (Parafungus)	200000	100000	200000	500000	166666.7
Garlic (*Allium sativum* L.)	200000	300000	100000	600000	200000.0
Gawed (*Piper betle*)	233000	400000	667000	1300000	433333.3
Table salt (.5 g/L H_20)	200000	100000	900000	1200000	400000
GRAND TOTAL				7634000	
GRAND MEAN					231333.3

APPENDIX TABLE 6. Effect of bacterial antagonists and plant extracts on the soil Population count of *F. oxysporum* f. sp. *chrysanthemi* (0 day before treatment) (transformed)

TREATMENT	REPLICATION I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	$2.0x10^5$	$6.0x10^5$	$5.0x10^5$	$13.0x10^5$	$4.3x10^5$
F.o.c. + Hot H_20 Treatment	$6.7x10^4$	$3.0x10^5$	$1.0x10^5$	$4.6x10^5$	$1.5x10^5$
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp.& *Flavobacterium* sp.	$2.0x10^5$	$1.0x10^5$	$1.0x10^5$	$4.0x10^5$	$1.3x10^5$
F. o.c + *Flavobacterium* sp.	$1.0x10^5$	$1.0x10^5$	$2.0x10^5$	$4.0x10^5$	$1.3x10^5$
F.o.c + *Pseudomonas* sp.	$6.0x10^5$	$1.0x10^5$	$3.0x10^5$	$10.0x10^5$	$3.3x10^5$
F.o.c. + *Flavobacterium* Sp. & *Pseudomonas* sp.	$2.0x10^5$	$6.7x10^4$	$2.0x10^5$	$4.6x10^5$	$1.5x10^5$
Mancozeb (Parafungus)	$2.0x10^5$	$1.0x10^5$	$2.0x10^5$	$5.0x10^5$	$1.6x10^5$
Garlic (*Allium sativum* L.)	$2.0x10^5$	$3.0x10^5$	$1.0x10^5$	$6.0x10^5$	$2.0x10^5$
Gawed (*Piper betle*)	$2.3x10^5$	$4.0x10^5$	$6.6x10^5$	$13.0x10^5$	$4.3x10^5$
Table salt (NaCl = .5g/L)	$2.0x10^5$	$1.0x10^5$	$9.0x10^5$	$12.0x10^5$	$4.0x10^5$
GRAND TOTAL				$76.3X10^5$	
GRAND MEAN					$2.3X10^5$

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Treatment	10	77.48	7.74	100.63	0.0001
Error	22	1.69	0.08		
Total	32	76.89			

** - highly significant at 1% level using DMRT CV= 5.77%

APPENDIX TABLE 7. Effect of bacterial antagonists and plant extracts on the soil population count of *F. oxysporum* f. sp. *chrysanthemi* 1 day after treatment (actual)

TREATMENT	REPLICATION I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	300000	500000	600000	1400000	466666.7
F.o.c. + Hot H_20	100000	200000	100000	400000	133333.3
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp +*Flavobacterium* sp.	200000	100000	133000	433000	144333.3
F. o.c +*Flavobacterium* sp.	100000	100000	167000	367000	122333.3
F.o.c + *Pseodomonas* sp.	500000	100000	200000	800000	2666666.7
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	100000	100000	200000	400000	133333.3
Mancozeb (Parafungus)	100000	100000	167000	367000	122333.3
Garlic (*Allium sativum* L.)	200000	200000	200000	600000	200000.0
Gawed (*Piper betle*)	200000	300000	600000	1100000	366666.7
Table salt (.5g/L H_20)	300000	100000	667000	1067000	355666.7
GRAND TOTAL				6934000	
GRAND MEAN					210121.2

APPENDIX TABLE 8. Effect of bacterial antagonists and plant extracts on the soil Population count of *F. oxysporum* f. sp. *chrysanthemi* 1 day after treatment (transformed)

TREATMENT	REPLICATION I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	$3.0x10^5$	$5.0x10^5$	$6.0x10^5$	$4.0x10^5$	$4.6x10^5$
F.o.c. + Hot H_20 Treatment	$1.0x10^5$	$2.0x10^5$	$1.0x10^5$	$4.0x10^5$	$1.3x10^5$
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp.& *Flavobacterium* sp.	$2.0x10^5$	$1.0x10^5$	$1.3x10^5$	$4.3x10^5$	$1.4x10^5$
F. o.c + *Flavobacterium* sp.	$1.0x10^5$	$1.0x10^5$	$1.6x10^5$	$3.6x10^5$	$1.2x10^5$
F.o.c + *Pseudomonas* sp.	$5.0x10^5$	$1.0x10^5$	$2.0x10^5$	$8.0x10^5$	$2.6x10^5$
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	$1.0x10^5$	$1.0x10^5$	$2.0x10^5$	$4.0x10^5$	$1.3x10^5$
Mancozeb (Parafungus)	$1.0x10^5$	$1.0x10^5$	$1.6x10^5$	$3.6x10^5$	$1.2x10^5$
Garlic (*Allium sativum* L.)	$2.0x10^5$	$2.0x10^5$	$2.0x10^5$	$6.0x10^5$	$2.0x10^5$
Gawed (Mancozeb)	$2.0x10^5$	$3.0x10^5$	$6.0x10^5$	$11.0x10^5$	$3.6x10^5$
Table salt (NaCl =.5g/L)	$3.0x10^5$	$1.0x10^5$	$6.6x10^5$	$10.6x10^5$	$3.5x10^5$
GRAND TOTAL				$69.3X10^5$	
GRAND MEAN					$2.1\ X10^5$

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Treatment	10	76.05	7.60	197.74	0.0001
Error	22	0.85	0.04		
Total	32	76.89			

** - highly significant at 1% level using DMRT CV= 4.13%

APPENDIX TABLE 9. Effect of bacterial antagonists and plant extracts on the soil population count of *F. oxysporum* f. sp. *chrysanthemi* 3 days after treatment (actual)

TREATMENT	REPLICATION I	II	III	TOTAL	MEAN
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	400000	600000	500000	1500000	500000.0
F.o.c. + Hot H_20	67000	167000	100000	334000	111333.3
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp + *Flavobacterium* sp.	100000	200000	100000	400000	133333.3
F. o.c + *Flavobacterium* sp.	100000	100000	133000	333000	111000.0
F.o.c + *Pseudomonas* sp.	400000	100000	200000	700000	233333.3
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	67000	100000	200000	367000	122333.3
Mancozeb (Parafungus)	67000	67000	100000	234000	78000.0
Garlic (*Allium sativum* L.)	200000	100000	200000	500000	166666.7
Gawed (*Piper betle*)	200000	200000	600000	1000000	333333.3
Table salt (.5g/L H_20)	200000	200000	600000	1000000	333333.3
GRAND TOTAL				6368000	
GRAND MEAN					192969.7

APPENDIX TABLE 10. Effect of bacterial antagonists and plant extracts on the soil population count of *F. oxysporum* f. sp. *chrysanthemi* 3 days after treatment (Transformed)

TREATMENT	REPLICATION I	II	II	TOTAL	MEAN
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	4.0×10^5	6.0×10^5	5.0×10^5	15.0×10^5	5.0×10^5
F.o.c. + Hot H_20 Treatment	6.7×10^4	1.6×10^5	1.0×10^5	3.3×10^5	1.1×10^5
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp.& *Flavobacterium* sp.	1.0×10^5	2.0×10^5	1.0×10^5	4.0×10^5	1.3×10^5
F.o.c + *Flavobacterium* sp.	1.0×10^5	1.0×10^5	1.3×10^5	3.33×10^5	1.1×10^5
F.o.c + *Pseudomonas* sp.	4.0×10^5	1.0×10^5	2.0x 105	7.0×10^5	2.3×10^5
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	6.7×10^4	1.0 x 105	2.0×10^5	3.6×10^5	1.2×10^5
Mancozeb (Parafungus)	6.7×10^4	6.7×10^4	1.0×10^5	2.3×10^4	7.8×10^4
Garlic (*Allium sativum* L.)	2.0×10^5	1.0 x 105	2.0×10^5	5.0×10^5	1.6×10^5
Gawed (*Piper betle*)	2.0×10^5	2.0x 105	6.0×10^5	10.0×10^5	3.3×10^5
Table salt (NaCl=5g/L)	2.0×10^5	2.0x 105	6.0×10^5	10.0×10^5	3.3×10^5
GRAND TOTAL				63.68×10^5	
GRAND MEAN					2.10×10^5

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Treatment	10	87.62	8.76	164.12	0.0001
Error	22	1.17	0.05		
Total	32	88.79			

** - highly significant at 1% level using DMRT CV= 4.59%

APPENDIX TABLE 11. Effect of bacterial antagonists and plant extracts on the soil population count of *F. oxysporum* f. sp. *chrysanthemi* 7 days after treatment (actual)

TREATMENT	REPLICATION I	II	III	TOTAL	MEAN
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	800000	1633000	933000	3366000	1122000.0
F.o.c. + Hot H_20	1100000	833000	1933000	3866000	1288667.0
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp + *Flavobacterium* sp.	500000	267000	100000	867000	289000.0
F. o.c + *Flavobacterium* sp.	200000	600000	967000	1767000	589000.0
F.o.c + *Pseodomonas* sp.	400000	200000	267000	867000	289000.0
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	167000	133000	133000	433000	144333.3
Mancozeb (Parafungus	67000	33000	100000	200000	66666.7
Garlic (*Allium sativum* L.)	600000	300000	900000	1800000	600000.0
Gawed (*Piper betle*)	200000	300000	600000	1100000	366666.7
Table salt (.5g/L H_20)	300000	200000	700000	1200000	400000.0
GRAND TOTAL				15466000	
GRAND MEAN					468666.7

APPENDIX TABLE 12. Effect of bacterial antagonists and plant extracts on the soil population count of *F. oxysporum* f. sp. *chrysanthemi* 7 days after treatment (Transformed)

TREATMENT	REPLICATION I	II	III	TOTAL	MEAN
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	8.0×10^5	16.33×10^5	9.33×10^5	33.66×10^5	11.22×10^5
F.o.c. + Hot H_20	11.0×10^4	8.33×10^5	19.3×10^5	38.66×10^3	12.88×10^5
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp + *Flavobacterium* sp.	5.0×10^5	2.67×10^5	1.0×10^5	8.67×10^5	58.9×10^5
F. o.c + *Flavobacterium* sp.	2.0×10^5	6.0×10^5	9.67×10^5	17.6×10^5	5.89×10^5
F.o.c + *Pseudomonas* sp.	4.0×10^5	2.0×10^5	2.67×10^5	8.67×10^5	2.89×10^5
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	1.67×10^5	1.33×10^5	1.33×10^5	4.33×10^5	1.44×10^5
Mancozeb (Parafungus)	6.7×10^4	3.3×10^4	1.0×10^5	2.0×10^5	6.66×10^4
Garlic (*Allium sativum* L.)	6.0×10^5	3.0×10^5	9.0×10^5	18.0×10^5	6.0×10^5
Gawed (*Piper betle*)	2.0×10^4	3.0×10^5	6.0×10^5	11.0×10^5	3.66×10^5
Table salt (.5g/L H_20)	3.0×10^5	2.0×10^5	6.0×10^5	12.0×10^5	4.0×10^5
GRAND TOTAL				154.66×10^5	
GRAND MEAN					46.86×10^5

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Treatment	10	87.62	8.76	164.12	0.0001
Error	22	1.17	0.05		
Total	32	88.79			

** - highly significant at 1% level using DMRT CV= 4.59%

APPENDIX TABLE 13 . Effect of bacterial antagonists and plant extracts on the soil population count of *F. oxysporum* f. sp. *chrysanthemi* 14 days after treatment ((actual)

TREATMENT	REPLICATION I	II	III	TOTAL	MEAN
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	600000	800000	500000	1900000	633333.3
F.o.c. + Hot H_20	400000	200000	167000	767000	255666.7
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp +*Flavobacterium* sp.	100000	200000	167000	467000	155666.7
F. o.c + *Flavobacterium* sp.	133000	100000	300000	533000	177666.7
F.o.c + *Pseudomonas* sp.	267000	133000	100000	500000	166666.7
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	133000	67000	100000	300000	100000.0
Mancozeb (Parafungus)	67000	33000	33000	133000	44333.30
Garlic (*Allium sativum* L.)	300000	200000	100000	600000	200000.0
Gawed (*Piper betle*)	900000	200000	333000	1433000	477666.7
Table salt (.5g/L H_20)	100000	200000	600000	900000	300000.0
Grand Total				7533000	
Grand Mean					228272.7

APPENDIX TABLE 14. Effect of bacterial antagonists and plant extracts on the soil population count of. *F. oxysporum* f. sp. chrysanthemi 14 days after treatment (transformed)

TREATMENT	REPLICATION I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0

Uninoculated-Treated	$6.0x10^5$	$8.0x10^5$	$5.0x10^5$	$19.0x10^5$	$6.3x10^5$
F.o.c. + Hot H_20 Treatment	$4.0x10^4$	$2.0x10^5$	$1.6x10^5$	$7.6x10^5$	$2.5x10^5$
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp.& *Flavobacterium* sp.	$1.0x10^5$	$2.0x10^5$	$1.6x10^5$	$4.6x10^5$	$1.5x10^5$
F. o.c + *Flavobacterium* sp.	$1.3x10^5$	$1.0x10^5$	$3.0x10^5$	$5.3x10^5$	$1.7x10^5$
F.o.c + *Pseudomonas* sp.	$2.6x10^5$	$1.3x10^5$	$1.0x10^5$	$5.0x10^5$	$1.6x10^5$
F.o.c. + *Flavobacterium* Sp. & *Pseudomonas* sp.	$1.3x10^5$	$6.7x10^4$	$1.0x10^5$	$3.0x10^5$	$1.0x10^5$
Mancozeb (Parafungus)	$6.7x10^4$	$3.3x10^4$	$3.3x10^4$	$1.3x10^5$	$4.4x10^4$
Garlic (*Allium sativum* L.)	$3.0x10^5$	$2.0x10^5$	$1.0x10^5$	$6.0x10^5$	$2.0x10^5$
Gawed (*Piper betle*)	$9.0x10^5$	$2.0x10^5$	$3.3x10^5$	$14.3x10^5$	$4.7x10^5$
Table salt (NaCl =.5g/L)	$1.0x10^5$	$2.0x10^5$	$6.0x10^5$	$9.0x10^5$	$3.0x10^5$
GRAND TOTAL				$75.3X10^5$	
GRAND MEAN					$2.2X10^5$

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Treatment	10	78.05	7.8	153.11	0.0001
Error	22	1.12	0.05		
Total	32	79.17			

** - highly significant at 1% level using DMRT CV= 4.73

APPENDIX TABLE 15. Effect of bacterial antagonists and plant extracts on the soil Population count of. *F. oxysporum* f. sp. chrysanthemi 21 days after treatment (actual)

TREATMENT	REPLICATION I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	

Uninoculated-Treated	400000	300000	300000	1000000	333333.3
F.o.c. + Hot H_20	300000	100000	100000	500000	166666.7
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp + *Flavobacterium* sp.	33000	67000	100000	200000	66666.7
F. o.c + *Flavobacterium* sp.	100000	100000	100000	300000	100000.0
F.o.c + *Pseodomonas* sp.	267000	300000	67000	634000	211333.3
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	100000	67000	67000	234000	78000.0
Mancozeb (Parafungus)	33000	33000	33000	99000	33000.0
Garlic (*Allium sativum* L.)	67000	33000	33000	133000	44333.3
Gawed (*Piper betle*)	300000	167000	100000	567000	189000.0
.5g Table salt/li H_20	100000	100000	300000	500000	166666.7
GRAND TOTAL				4167000	
GRAND MEAN					126272.7

APPENDIX TABLE 16. Effect of bacterial antagonists and plant extracts on the soil population count of. *F. oxysporum* f. sp. chrysanthemi 21 days after treatment (transformed)

	REPLICATION				
TREATMENT	I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	$4.0x10^5$	$3.0x10^5$	$3.0x10^5$	$10.0x10^5$	$3.3x10^5$

F.o.c. + Hot H_20 Treatment	$3.0x10^5$	$1.0x10^5$	$1.0x10^5$	$5.0x10^5$	$1.6x10^5$
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp.& *Flavobacterium* sp.	$3.3x10^4$	$6.7x10^4$	$1.0x10^5$	$2.0x10^5$	$6.6x10^4$
F. o.c + *Flavobacterium* sp.	$1.0x10^5$	$1.0x10^5$	$1.0x10^5$	$3.0x10^5$	$1.0x10^5$
F.o.c + *Pseudomonas* sp.	$2.6x10^5$	$3.0x10^5$	$6.7x10^4$	$2.3x10^5$	$2.1x10^5$
F.o.c. + *Flavobacterium* Sp. & *Pseudomonas* sp.	$1.0x10^5$	$6.7x10^4$	$6.7x10^4$	$2.3x10^5$	$7.8x10^4$
Mancozeb (Parafungus)	$3.3x10^4$	$3.3x10^4$	$3.3x10^4$	$9.9x10^4$	$3.3x10^4$
Garlic (*Allium sativum* L.)	$6.7x10^4$	$3.3x10^4$	$3.3x10^4$	$1.3x10^5$	$4.4x10^4$
Gawed (*Piper betle*)	$3.0x10^5$	$1.6x10^5$	$1.0x10^5$	$5.6x10^5$	$1.8x10^5$
Table salt (NaCl =.5g/L)	$1.0x10^5$	$1.0x10^5$	$3.0x10^5$	$5.0x10^5$	$1.6x10^5$
GRAND TOTAL				$41.6X10^5$	
GRAND MEAN					$1.2X10^5$

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Treatment	10	71.11	7.11	175.41	0.0001
Error	22	0.89	0.04		
Total	32	72.00			

** - highly significant at 1% level using DMRT CV= 4.41%

APPENDIX TABLE 17. Effect of bacterial antagonists and plant extracts on the soil population count of. *F. oxysporum* f. sp. chrysanthemi 28 days after treatment (actual)

TREATMENT	REPLICATION I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated	100000	67000	133000	300000	100000.0

F.o.c. + Hot H_20	33000	33000	33000	99000	33000.0
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp + *Flavobacterium* sp.	33000	33000	0	66000	22000.0
F. o.c + *Flavobacterium* sp.	33000	33000	33000	99000	33000.0
F.o.c + *Pseodomonas* sp.	167000	33000	33000	233000	77666.7
F.o.c. + *Flavobacterium* sp. & *Pseudomonas* sp.	33000	33000	33000	99000	33000.0
Mancozeb (Parafungus)	33000	0	0	33000	11000.0
Garlic (*Allium sativum* L.)	67000	0	0	67000	22333.3
Gawed (*Piper betle*)	33000	33000	0	66000	22000.0
Table salt (.5g/L H_20	33000	0	100000	133000	44333.3
GRAND TOTAL				1195000	
GRAND MEAN					36212.12

APPENDIX TABLE 18. Effect of bacteria l antagonists and plant extracts on the soil population count of *F. oxysporum* f. sp. chrysanthemi 28 days after treatment (transformed)

	REPLICATION				
TREATMENT	I	II	III	Total	Mean
Uninoculated-Untreated	0.0	0.0	0.0	0.0	0.0
Uninoculated-Treated F.o.c. + Hot H_20	$1.0x10^5$	$6.7x10^4$	$1.3x10^5$	$3.0x10^5$	$1.0x10^5$

Treatment					
	$3.3x10^4$	$3.3x10^4$	$3.3x10^4$	$9.9x10^4$	$3.3.x10^4$
F. o. c. + Hot H_20, after 1 week add garlic, after 10 days add *Pseudomonas* sp.& *Flavobacterium* sp.	$3.3x10^4$	$3.3x10^4$	0.0	$6.6x10^4$	$2.2x10^4$
F. o.c + *Flavobacterium* sp.	$3.3x10^4$	$3.3x10^4$	$3.3x10^4$	$9.9x10^4$	$3.3x10^4$
F.o.c + *Pseudomonas* sp.	$1.6x10^5$	$3.3x10^4$	$3.3x10^4$	$2.3x10^5$	$7.7x10^4$
F.o.c. + *Flavobacterium* Sp. & *Pseudomonas* sp.	$3.3x10^4$	$3.3x10^4$	$3.3x10^4$	$9.9x10^4$	$3.3x10^4$
Mancozeb (Parafungus)	$3.3x10^4$	0.0	0.0	$3.3x10^4$	$1.1x10^4$
Garlic (*Allium sativum* L.)	$6.7x10^4$	0.0	0.0	$6.7x10^4$	$2.2x10^4$
Gawed (*Piper betle*)	$3.3x10^4$	$3.3x10^4$	0.0	$6.6x10^4$	$228x10^4$
Table salt (NaCl =.5g/L)	$3.3x10^4$	0.0	$1.0x10^5$	$1.3x10^5$	$4.4x10^4$
GRAND TOTAL				$11.9X10^5$	
GRAND MEAN					$3.6X10^4$

ANOVA TABLE

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Treatment	10	79.53	7.95	2.43	0.0396
Error	22	71.96	3.29		
Total	32	151.49			

* - significant at 5% level using DMRT CV= 55.87%

APPENDIX TABLE 19. Effect of bacterial antagonists and plant extracts on Fusarium wilt infection of chrysanthemum (1st week)

TREATMENT	REPLICATION I	II	III	IV	TOTAL	MEAN
Uninoculated – Untreated	1.00	1.00	1.00	1.00	4.00	1.00
Uninoculated – Treated	2.33	2.00	2.33	2.00	8.66	2.17
F.o.c + Hot H_20 + garlic extract (1 week after) + comb. of *Pseudomonas* sp. and *Flavobacterium* sp. after 17 days	1.67	1.67	1.33	1.33	6.00	1.50
Comb. of *Flavobacterium* sp. and *Pseudomonas* sp.	2.00	2.00	1.67	2.00	7.67	1.92
Mancozeb (Parafungus)	1.67	1.33	1.33	1.33	5.66	1.42
Thiopanate methyl (Fungitox)	1.33	1.67	1.00	1.00	5.00	1.25
Garlic (*Allium sativum* L.)	2.33	2.00	1.67	1.67	7.67	1.92
Gawed (Piperbetle)	2.33	2.00	2.33	2.33	8.99	2.25
Table salt (1g/l H_20)	1.67	2.00	1.67	2.33	7.67	1.92
GRAND TOTAL					66.32	
GRAND MEAN						1.51

ANOVA

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Model	35	21.85	0.62	3.06	0.0001
Treatment	8	17.69	2.21	14.33	0.0001
Rep(TRT)	27	4.17	0.15	0.76	0.7875
Error	72	14.67	0.2		
TOTAL	107	36.52			

** Highly significant at 1% level using DMRT CV= 26.49%

APPENDIX TABLE 20. Effect of bacterial antagonists and plant extracts on the fusarium wilt infection of chrysanthemum (2^{nd} week)

TREATMENT	REPLICATION I	II	III	IV	TOTAL	MEAN
Uninoculated – Untreated	1.00	1.00	1.00	1.00	4.00	1.00
Uninoculated – Treated	3.33	3.00	3.00	3.00	12.33	3.082
F.o.c + Hot H_20 + garlic extract (1 week after) + comb. of *Pseudomonas* sp. and *Flavobacterium* sp. after 17 days	2.67	3.00	3.00	3.00	11.67	2.92
Comb. of *Flavobacterium* sp. and *Pseudomonas* sp.	2.00	1.67	2.00	2.00	7.67	2.33
Mancozeb (Parafungus)	1.67	2.33	1.33	1.67	7.00	1.92
Thiopanate methyl (Fungitox)	2.00	2.33	1.67	2.67	8.67	1.75
Garlic (*Allium sativum* L.)	2.67	2.67	3.33	1.67	10.34	2.17
Gawed (Piperbetle)	2.33	2.00	2.33	2.33	8.99	2.59
Table salt (1g/l H_20)	2.00	2.00	2.00	2.67	8.67	2.25
GRAND TOTAL						2.17
GRAND MEAN					2.23	

ANOVA

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Model	35	48.55	1.39	6.81	0.0001
Treatment	8	39.96	4.99	15.71	0.0001
Rep(TRT)	27	8.58	0.32	1.56	0.0696
Error	72	14.67	0.2		
TOTAL	107	63.21			

** Highly significant at 1% level using DMRT CV= 20.22%

APPENDIX TABLE 21. Effect of bacterial antagonist and plant extracts on fusarium wilt infection of chrysanthemum (3rd week)

TREATMENT	REPLICATION I	II	III	IV	TOTAL	MEAN
Uninoculated – Untreated	1.00	1.00	1.00	1.00	4.00	1.00
Uninoculated – Treated	3.67	4.00	3.67	3.67	15.01	3.7525
F.o.c + Hot H_20 + garlic extract (1 week after) + comb. of *Pseudomonas* sp. and *Flavobacterium* sp. after 17 days	3.67	3.33	4.00	4.00	15.00	3.75
Comb. of *Flavobacterium* sp. and *Pseudomonas* sp.	3.00	3.33	4.00	2.33	12.66	3.165
Mancozeb (Parafungus)	2.00	2.67	2.00	2.00	8.67	2.1675
Thiopanate methyl (Fungitox)	2.00	2.33	2.00	2.00	8.33	2.0825
Garlic (*Allium sativum* L.)	2.00	3.33	2.67	3.33	11.33	2.8325
Gawed (Piperbetle)	3.00	3.33	3.67	3.67	13.67	3.4175
Table salt (1g/l H_20)	3.00	3.00	2.00	3.33	11.33	2.8325
GRAND TOTAL	18.67	21.32	20.34	20.66	80.99	20.2475
GRAND MEAN						

ANOVA

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Model	35	92	2.63	7.1	0.0001
Treatment	8	77.67	9.71	18.29	0.0001
Rep (TRT)	27	14.33	0.53	1.43	0.1153
Error	72	26.67	0.37		
TOTAL	107	118.67			

** Highly significant at 1% level using DMRT CV= 21.91%

APPENDIX TABLE 22. Effect of bacterial antagonist and plant extracts on fusarium wilt infection of chrysanthemum (4th week)

TREATMENT	REPLICATION I	II	III	IV	TOTAL	MEAN
Uninoculated – Untreated	1.00	1.00	1.00	1.00	4.00	1.00
Uninoculated – Treated	4.00	4.67	4.00	4.33	17.00	4.25
F.o.c + Hot H_20 + garlic extract (1 week after) + comb. of *Pseudomonas* sp. and *Flavobacterium* sp. after 17 days	3.67	4.00	4.33	4.00	16.00	4.00
Comb. of *Flavobacterium* sp. and *Pseudomonas* sp.	3.33	3.33	4.33	2.33	13.32	3.33
Mancozeb (Parafungus)	2.33	2.67	2.00	2.00	9.00	2.25
Thiopanate methyl (Fungitox)	2.00	2.33	2.00	2.00	8.66	2.17
Garlic (*Allium sativum* L.)	2.33	4.00	2.67	4.00	12.67	3.17
Gawed (Piperbetle)	2.00	3.67	4.00	3.67	14.67	3.67
Table salt (1g/l H_20)	3.33	3.33	2.00	4.33	12.99	3.25
GRAND TOTAL					3.33	
GRAND MEAN						3.01

ANOVA

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Model	35	128.32	3.67	6.49	0.0001
Treatment	8	101.57	12.7	12.82	0.0001
Rep(TRT)	27	26.75	0.99	1.75	0.031
Error	72	40.67	0.56		
TOTAL	107	168.99			

** Highly significant at 1% level using DMRT CV= 24.97%

APPENDIX TABLE 23. Effect of bacterial antagonist and plant extracts on fusarium wilt infection of chrysanthemum (5th week)

TREATMENT	REPLICATION I	II	III	IV	TOTAL	MEAN
Uninoculated – Untreated	1.00	1.00	1.00	1.00	4.00	1.00
Uninoculated – Treated	4.00	4.67	4.33	4.67	17.67	4.42
F.o.c + Hot H_20 + garlic extract (1 week after) + comb. of *Pseudomonas* sp. and *Flavobacterium* sp. after 17 days	4.00	4.00	4.67	4.67	17.34	4.36
Comb. of *Flavobacterium* sp. and *Pseudomonas* sp.	3.33	5.00	2.33	2.33	12.99	3.25
Mancozeb (Parafungus)	2.33	2.67	2.00	2.33	9.33	2.33
Thiopanate methyl (Fungitox)	2.33	2.67	2.00	2.00	9.00	2.25
Garlic (*Allium sativum* L.)	2.00	4.67	2.67	4.33	13.67	3.42
Gawed (Piperbetle)	4.00	4.33	4.33	3.67	16.33	4.08
Table salt (1g/l H_20)	3.67	3.67	2.00	4.67	14.01	3.50
GRAND TOTAL						
GRAND MEAN						

ANOVA

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Model	35	164.85	4.71	5.98	0.0001
Treatment	8	123.18	15.4	9.98	0.0001
Rep(TRT)	27	41.67	1.54	1.96	0.0125
Error	72	56.67	0.78		
TOTAL	107	221.52			

** Highly significant CV= 27.69%

APPENDIX TABLE 24. Effect of bacterial antagonist and plant extracts on fusarium wilt infection of chrysanthemum (6^{th} week)

TREATMENT	REPLICATION I	II	III	IV	TOTAL	MEAN
Uninoculated – Untreated	1.00	1.00	1.00	1.00	4.00	1.00
Uninoculated – Treated	5.33	5.00	4.67	4.67	19.67	4.92
F.o.c + Hot H_20 + garlic extract (1 week after) + comb. of *Pseudomonas* sp. and *Flavobacterium* sp. after 17 days	5.00	4.67	5.33	5.00	20.00	5.00
Comb. of *Flavobacterium* sp. and *Pseudomonas* sp.	4.33	4.00	5.00	2.33	15.66	3.92
Mancozeb (Parafungus)	2.33	2.67	2.00	2.00	9.00	2.25
Thiopanate methyl (Fungitox)	2.33	2.67	2.00	2.00	9.00	2.25
Garlic (*Allium sativum* L.)	2.00	4.67	2.67	4.33	13.67	3.42
Gawed (Piperbetle)	4.00	4.00	5.00	3.67	16.67	4.17
Table salt (1g/l H_20)	3.67	3.67	2.00	4.67	14.01	3.50
GRAND TOTAL						
GRAND MEAN						3.38

ANOVA

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Model	35	213.43	6.10	4.88	0.0001
Treatment	8	169.52	21.19	13.03	0.0001
Rep(TRT)	27	43.92	1.63	1.30	0.1882
Error	72	90.00	1.25		
TOTAL	107	303.43			

** Highly significant at 1% level using DMRT CV= 33.08%

APPENDIX TABLE 25. Effect of bacterial antagonists and plant extracts on Fusarium wilt infection of chrysanthemum (7^{th} week)

TREATMENT	REPLICATION I	II	III	IV	TOTAL	MEAN
Uninoculated – Untreated	1.00	1.00	1.00	1.00	4.00	1.00
Uninoculated – Treated	5.33	5.33	5.67	4.67	21.00	5.25
F.o.c + Hot H_20 + garlic extract (1 week after) + comb. of *Pseudomonas* sp. and *Flavobacterium* sp. after 17 days	6.00	6.00	5.33	5.00	22.33	5.58
Comb. of *Flavobacterium* sp. and *Pseudomonas* sp.	4.33	4.00	5.33	2.33	15.99	4.00
Mancozeb (Parafungus)	2.33	2.67	2.00	2.67	9.67	2.42
Thiopanate methyl (Fungitox)	3.33	2.67	2.00	2.00	10.00	2.50
Garlic (*Allium sativum* L.)	2.00	4.00	2.67	4.33	13.67	3.42
Gawed (Piperbetle)	4.67		5.00	4.67	18.34	4.59
Table salt (1g/l H_20)	4.00	4.00	2.00	5.00	15.00	3.75
GRAND TOTAL					130.00	
GRAND MEAN						3.61

ANOVA

Source of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	F Value	Probability
Rep(TRT)	8	68.82	8.60	13.11	0.0001
Error	27	17.72	0.66		
TOTAL	35	86.54			

** Highly significant at 1% level using DMRT CV= 22.43%

APPENDIX TABLE 26. Effect of bacterial antagonists and plant extracts on Vascular discoloration (cm) on chrysanthemum

TREATMENTS	REPLICATION					
	I	II	III	IV	TOTAL	MEAN
Uninoculated/Untreated	0.00	0.00	0.00	0.00	0.00	0.00
Uninoculated /Treated	5.00	5.33	8.17	13.83	32.33	8.08
Hot H_20 treatment + garlic extract (1 week after) + comb. Of *Pseudomonas* ap. & *Flavobacterium* sp after 17 days	7.17	12.67	10.33	5.33	35.50	8.88
Comb. of *Pseudomonas* sp. & *Flavobacterium* sp.	2.67	3.50	3.67	1.67	11.51	2.88
Mancozeb (Parafungus)	3.67	0.67	2.17	2.33	8.84	2.21
Thiophanate methyl (Fungitox)	0.67	0.17	3.50	1.00	5.34	1.34
Garlic extract (*Allium sativum* L)	1.33	3.67	3.50	2.67	11.17	2.79
Gawed extract (*Piper betle*)	2.67	4.17	5.67	3.67	16.18	4.05
Table Salt (1g/L H_20)	0.67	2.67	2.07	6.67	12.08	3.02
GRAND TOTAL					132.95	
GRAND MEAN						3.69

ANOVA

Sources of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	Computed F	Probability
Treatment	8	278.29	34.79	7.59	0.0001
Error	27	123.73	4.58		
TOTAL	35	402.02			
** -highly significant at 1% level using DMRT				CV	57.96%

APPENDIX TABLE 27. Effect of bacterial antagonists and plant extracts on fresh top weight (g) of chrysanthemum plant

TREATMENTS	REPLICATION				TOTAL	MEAN
	I	II	III	IV		
Uninoculated/Untreated	34.00	34.33	34.00	34.33	136.66	34.17
Uninoculated /Treated	18.33	19.67	38.33	36.33	112.66	28.17
Hot H_20 treatment + garlic extract (1 week after) + comb. Of *Pseudomonas* ap. & *Flavobacterium* sp after 17 days	5.00	3.00	0.00	7.67	15.67	3.92
Comb. of *Pseudomonas* sp. & *Flavobacterium* sp.	37.33	43.67	49.67	47.33	178.00	44.50
Mancozeb (Parafungus)	45.33	41.67	41.67	42.67	171.34	42.84
Thiophanate methyl (Fungitox)	26.67	46	58.00	43.67	174.34	43.59
Garlic extract (*Allium sativum* L)	41.67	48.67	35.00	44.67	170.01	42.50
Gawed extract (*Piper betle*)	30.67	33.00	37.33	6.00	107.00	26.75
Table Salt (.5g/L H_20)	41.33	45.33	50.67	31.00	168.33	42.08
GRAND TOTAL					1234.01	
GRAND MEAN						34.28

ANOVA

Sources of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	Computed F	Probability
Treatment	8	5634.79	704.35	10.17	0.0001
Error	27	1870.31	69.27		
TOTAL	35	7505.10			

** -highly significant at 1% level using DMRT CV 24.28%

APPENDIX TABLE 28. Effect of bacterial antagonists and plant extracts on the Fresh root weight (g) of chrysanthemum plant

TREATMENTS	REPLICATION					
	I	II	III	IV	TOTAL	MEAN
Uninoculated/Untreated	5.73	6.50	6.70	4.77	23.70	5.93
Uninoculated /Treated	2.40	2.63	1.23	4.23	10.49	2.62
Hot H_20 treatment + garlic extract (1 week after) + comb. Of *Pseudomonas* ap. & *Flavobacterium* sp. after 17 days)	1.50	0.73	0.67	0.67	3.57	0.89
Comb. of *Pseudomonas* sp. & *Flavobacterium* sp.	6.37	7.03	7.13	5.10	25.63	6.41
Mancozeb (Parafungus)	4.10	5.23	6.63	3.63	19.59	4.90
Thiophanate methyl (Fungitox)	4.33	6.03	5.53	7.47	23.36	5.84
Garlic extract (*Allium sativum* L)	5.23	5.63	4.47	4.73	20.06	5.02
Gawed extract (*Piper betle*)	4.23	5.50	3.57	2.80	16.10	4.03
Table Salt (.5g/L H_20)	4.01	5.83	6.03	2.67	118.63	4.66
Grand Total					130	
Grand Mean						3.61

ANOVA

Sources of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	Computed F	Probability
Treatment	8	98.69	12.34	10.20	0.0001
Error	27	32.66	1.21		
Total	35	131.35			

** -highly significant at 1% level using DMRT CV 24.57%

APPENDIX TABLE 29. Quality cutflower (Long)

TREATMENTS	REPLICATION					
	I	II	III	IV	TOTAL	MEAN
Uninoculated/Untreated	0.00	0.00	0.00	0.00	0.00	0.00
Uninoculated /Treated	0.00	0.00	0.00	0.00	0.00	0.00
Hot H_20 treatment + garlic extract (1 week after) + comb. of *Pseudomonas* ap. & *Flavobacterium* sp after 17 DAT)	0.00	0.00	0.00	0.00	0.00	0.00
Comb. of *Pseudomonas* sp. & *Flavobacterium* sp.	0.00	1.00	0.00	1.00	2.00	0.50
Mancozeb (Parafungus)	0.00	0.00	2.00	2.00	4.00	1.00
Thiophanate methyl (Fungitox)	0.00	1.00	2.00	1.00	4.00	1.00
Garlic extract (*Allium sativum* L)	0.00	0.00	1.00	0.00	1.00	0.25
Gawed extract (*Piper betle*)	0.00	0.00	0.00	0.00	0.00	0.00
Table Salt (.5g/L H_20)	0.00	0.00	1.00	0.00	1.00	0.25
Grand Total					12.00	
Grand Mean						0.33

ANOVA

Sources of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	Computed F	Probability
Treatment	8	5.50	0.09	2.18	0.0618
Error	27	8.50	0.32		
Total	35	14.00			

* - significant at 5% level using DMRT CV 168.327%

APPENDIX TABLE 30. Quality cutflower (Medium)

TREATMENTS	REPLICATION					
	I	II	III	IV	TOTAL	MEAN
Uninoculated/Untreated	3.00	3.00	3.00	3.00	12.00	3.00
Uninoculated /Treated	0.00	0.00	0.00	1.00	1.00	0.25
Hot H_20 treatment + garlic extract (1 week after) + comb. of *Pseudomonas* ap. & *Flavobacterium* sp after 17 days	0.00	0.00	0.00	0.00	0.00	0.00
Comb. of *Pseudomonas* sp. & *Flavobacterium* sp.	1.00	1.00	1.00	1.00	4.00	1.00
Mancozeb (Parafungus)	0.00	0.00	1.00	1.00	2.00	0.50
Thiophanate methyl (Fungitox)	1.00	0.00	1.00	1.00	3.00	0.75
Garlic extract (*Allium sativum* L)	2.00	0.00	1.00	1.00	4.00	1.00
Gawed extract (*Piper betle*)	1.00	1.00	1.00	0.00	3.00	0.75
Table Salt (.5g/L H_20)	2.00	1.00	1.00	0.00	4.00	1.00
Grand Total					33.00	
Grand Mean						0.92

ANOVA

Sources of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	Computed F	Probability
Treatment	8	23.50	2.94	10.94	0.0001
Error	27	7.25	0.27		
Total	35	30.75			
** -highly significant at 1% level using DMRT				CV	56.53%

APPENDIX TABLE 31. Quality cutflower (Short)

TREATMENTS	REPLICATION					
	I	II	III	IV	TOTAL	MEAN
Uninoculated/Untreated	0.00	0.00	0.00	0.00	0.00	0.00
Uninoculated /Treated	1.00	1.00	0.00	0.00	2.00	0.50
Hot H_20 treatment + garlic extract (1 week after) + comb. of *Pseudomonas* ap. & *Flavobacterium* sp after 17 days	0.00	0.00	0.00	0.00	0.00	0.00
Comb. of *Pseudomonas* sp. & *Flavobacterium* sp.	0.00	1.00	1.00	0.00	2.00	0.50
Mancozeb (Parafungus)	2.00	2.00	0.00	0.00	4.00	1.00
Thiophanate methyl (Fungitox)	1.00	1.00	0.00	1.00	3.00	0.75
Garlic extract (*Allium sativum* L)	1.00	1.00	1.00	0.00	3.00	0.75
Gawed extract (*Piper betle*)	0.00	1.00	1.00	1.00	3.00	0.75
Table Salt (.5g/L H_20)	1.00	1.00	1.00	0.00	3.00	0.75
Grand Total					20.00	
Grand Mean						0.56

ANOVA

Sources of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	Computed F	Probability
Treatment	8	3.89	0.49	1.46	0.2156
Error	27	9.00	0.33		
Total	35	12.89			
* - significant at 5% level using DMRT				CV	103.92%

APPENDIX TABLE 32. Quality cutflower (CB/Reject)

TREATMENTS	REPLICATION					
	I	II	III	IV	TOTAL	MEAN
Uninoculated/Untreated	0.00	0.00	0.00	0.00	0.00	0.00
Uninoculated /Treated	2.00	2.00	3.00	2.00	9.00	2.25
Hot H_20 treatment + garlic extract (1 week after) + comb. of *Pseudomonas* ap. & *Flavobacterium* sp after 17days	3.00	3.00	3.00	3.00	12.00	3.00
Comb. of *Pseudomonas* sp. & *Flavobacterium* sp.	2.00	0.00	1.00	1.00	4.00	1.00
Mancozeb (Parafungus)	1.00	1.00	0.00	0.00	2.00	0.50
Thiophanate methyl Fungitox)	1.00	1.00	0.00	0.00	2.00	0.50
Garlic extract (*Allium sativum* L)	0.00	2.00	0.00	2.00	4.00	1.00
Gawed extract (*Piper betle*)	2.00	1.00	1.00	2.00	6.00	1.50
Table Salt (.5g/L H_20)	0.00	1.00	0.00	3.00	4.00	1.00
GRAND TOTAL					43.00	
GRAND MEAN						1.19

ANOVA

Sources of Variation	Degree of Freedom	Sum of Squares	Mean of Squares	Computed F	Probability
Treatment	8	27.89	3.49	5.98	0.0002
Error	27	15.75	0.58		
TOTAL	35	43.64			

** - highly significant at 1% level using DMRT CV 27.89%

APPENDIX TABLE 33. Effect of bacterial antagonists and plant extracts on cutflower yield.[a]

TREATMENT	CLASSIFI CATION				
	LONG	MEDIUM	SHORT	CB	NM
Uninoculated/Untreated	0.00b	3.00a	0.00b	0.00b	0.00c
Uninoculated /Treated	0.00b	0.25bc	0.50ab	0.00b	2.25ab
Hot H_20 treatment + garlic extract (1 week after) + comb. Of *Pseudomonas* ap. & *Flavobacterium* sp (after 17 DAT)	0.00b	0.00c	0.00b	0.00b	3.00a
Comb. Of *Pseudomonas* sp. & *Flavobacterium* sp.	0.50b	0.00c	0.00b	0.50a	0.50bc
Parafungus (Mancozeb)	1.00a	0.50bc	1.00a	0.50a	0.00c
Fungitox (Thiophanate methyl)	1.00a	0.75bc	0.75ab	0.50a	0.00c
Garlic extract (*Allium sativum* L)	0.25ab	1.00b	0.75ab	0.50a	0.00c
Gawed extract (*Piper betle*)	0.00b	0.75bc	0.75ab	0.50a	0.50bc
Table Salt (.5g/L H_20)	0.25ab	1.00b	0.75ab	0.25ab	0.75b
Grand Total					
CV	168.32%	56.53%	103.92%	63.94 %	63.94%

[a]Data are means of four replications. Means followed by a common letter are not significantly different at 5% level using DMRT

APPENDIX TABLE 34 . Effect of bacterial antagonists and plant extracts on cutflower yield.[a]

TREATMENT	CLASSIFICATION/GROSS SALE (PhP)					
	Long	Medium	Short	CB	Reject	Total
Uninoculated/Untreated	0.00	60.00	0.00	0.0	0.00	P60.00
Uninoculated /Treated	0.00	5.00	8.34	0.00	0.00	P13.84
Hot H_20 treatment + garlic extract (1 week after) + comb. Of *Pseudomonas* ap. & *Flavobacterium* sp (after 17 DAT)	0.00	0.00	0.00	0.00	0.00	0.00
Comb. Of *Pseudomonas* sp. & *Flavobacterium* sp.	11.66	20.00	8.34	10.00	0.00	50.00
Parafungus (Mancozeb)	23.32	10.00	16.68	5.00	0.00	55.00
Fungitox (Thiophanate methyl)	23.32	15.00	12.51	5.00	0.00	55.83
Garlic extract (*Allium sativum* L)	5.83	20.00	12.51	10.00	0.00	48.34
Gawed extract (*Piper betle*)	0.00	15.00	12.51	10.00	0.00	37.51
Table Salt (.5g/L H_20)	5.83	20.00	12.51	2.50	0.00	40.84
GRAND TOTAL						
GRAND MEAN						

[a]Data are means of four replications.