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Root-knot Nematode Problem of Some Winter Ornamental Plants and Its Biomanagement

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Abstract: A microplot study under field conditions was carried out during 2 consecutive years to assess the effect of root-knot nematode infection (2,000 *Meloidogyne incognita* eggs/kg soil) on three winter ornamental plants: hollyhock (*Althea rosea*), petunia (*Petunia hybrida*), and poppy (*Papaver rhoeas*). Effects of root-dip treatment with the biocontrol agents *Pochonia chlamydosporia, Bacillus subtilis,* and *Pseudomonas fluorescens* and the nematicide fenamiphos were tested. The three ornamental species were highly susceptible to *M. incognita,* developing 397 and 285 (hollyhock), 191 and 149 (petunia), and 155 and 131 (poppy) galls and egg masses per root system, respectively, and exhibited 37% (petunia), 29% (poppy), and 23% (hollyhock) ($P = 0.05$) decrease in the flower production. Application of fenamiphos, *P. chlamydosporia, P. fluorescens,* and *B. subtilis* suppressed nematode pathogenesis (galls + egg masses) by 64%, 37%, 27%, and 24%, respectively, leading to 14% to 29%, 7% to 15%, 14% to 36%, and 7% to 33% increase in the flower production of the ornamental plants, respectively. Treatment with *P. fluorescens* also increased the flowering of uninfected plants by 11% to 19%. Soil population of *M. incognita* was decreased ($P = 0.05$) due to various treatments from 2 months onward, being greatest with fenamiphos, followed by *P. chlamydosporia, B. subtilis,* and *P. fluorescens.* Frequency of colonization of eggs, egg masses, and females by the bioagents was greatest by *P. chlamydosporia,* i.e., 25% to 29%, 47% to 60%, and 36% to 41%, respectively. Colonization of egg masses by *B. subtilis* and *P. fluorescens* was 28% to 31% and 11% to 13%, respectively, but the frequency was 0.3% to 1.3% in eggs. Rhizosphere population of the bioagents was increased ($P = 0.05$) over time, being usually greater in the presence of nematode.

Key words: Bacillus subtilis, biocontrol, hollyhock, *Meloidogyne incognita,* petunia, *Pochonia chlamydosporia,* poppy, *Pseudomonas fluorescens,* rhizosphere population.

Root-knot nematodes, *Meloidogyne* species, are serious pests of an array of agricultural crops and cause tremendous yield loss to them, particularly under subtropical and tropical climates (Sasser, 1979). Seasonal ornamentals are a major group of plants that are highly susceptible to root-knot nematodes and exhibit considerable yield loss (Khan et al., 1987; Sasser, 1989). Effects of root-knot nematodes on crop damage and loss to flower production of ornamental plants have not been adequately studied. The demand for flowers in India for various social and religious purposes, medicinal use, and export has greatly increased during the last two decades. As a result, floriculture is emerging as an attractive business (Raghava and Dadlani, 1999).

The bulk of farmers engaged in commercial floriculture in India and other developing countries is small, and growing flowers is a small segment of their traditional farming system. These farmers are illiterate and lack systematic approaches toward farming. Hence, attack by root-knot nematodes on ornamental plants remains unrecognized. Sometimes plants are left intact in fields for weeks after flower production ceases, allowing the nematode to complete its life cycle and resulting in population buildup.

If diseases caused by nematodes are recognized, farmers do not apply a nematicide because of the high cost and phytotoxicity to ornamental plants (Khan and Khan, 1989). In view of increasing domestic needs and greater export potential in floriculture, it is essential to evaluate the effect of root-knot nematode infection on the productivity of ornamental plants and possible biomanagement of the disease. This investigation deals with a microplot study to estimate the loss of flower production caused by the root-knot nematode *M. incognita* to hollyhock, petunia, and poppy and to evaluate biocontrol through root-dip treatment with *Pochonia chlamydosporia* (= *Verticillium chlamydosporium*), *Bacillus subtilis,* and *Pseudomonas fluorescens.*

Materials and Methods

Nematode inoculation: Inoculum of *M. incognita* (Kofoid & White) Chitwood was prepared from egg masses, which were collected from egg plant, *Solanum melongena* L. cv PPR, growing in a pure culture (*M. incognita*) bed. Roots were gently washed with running tap water and cut into 2-cm to 4-cm pieces. The root pieces were shaken vigorously in 0.52% NaOCl for 2 to 3 minutes in a conical flask. The solution was quickly poured over a 75-µm-pore and 26-µm-pore sieve and rinsed several times with water to remove NaOCl residues. The eggs were collected from the 26-µm-pore sieve (Hussey and Barker, 1973). The egg suspension was standardized to 2,000 eggs/liter of water. The nematode suspension was added to soil throughout the microplot at 2,000 eggs/kg soil (320 locations, 0.64 million eggs/microplot), and nematodes were mixed into the soil at 15-cm depth. Inoculation was performed 1 day prior to planting of seedlings. The higher egg inoculum was applied because 70% to 80% of the eggs hatched in the soil. This was determined by inoculating 2 kg autoclaved and field soil with 4,000 eggs in clay pots separately. After 2 weeks hatched juveniles were estimated using Cobb's decanting and sieving method. A check without egg inoculation was also maintained. Root-knot nematode populations in Indian fields with susceptible

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crops vary from 700 to more than 2,100 J2/kg soil depending on age of crop and temperature (Khan, 1997).

Application of bioagents: Mass culture of *Pochonia chlamydosporia* (=*Verticillium chlamydosporium*) (Goodard) Zare and Gams, *Bacillus subtilis* Cohn *amend.* Prazmowski, and *Pseudomonas fluorescens* (Threvesan) Migula were prepared in conical flasks on potato carrot broth, nutrient broth (NB), and Kings-B supplemented (KBS) media, respectively. The KBS was prepared by adding 75 mg cycloheximide and penicillin and 45 mg novobiocin/liter to Kings-B medium to make it selective for *P. fluorescens* (Sands and Rovira, 1970). To determine minimum inhibitory concentration (MIC) of bacterial strains, antibiotic sensitivity tests were performed using amoxicillin, chloramphenicol, cloxacillin, co-trimoxazole, doxycillin hydrochloride, flucanazole, methacillin, nalidixic acid, nitrofurantoin, novobiocin, penicillin, and tetracycline (Bauer et al., 1966). The antibiotic profiling revealed 500 µg/20 ml medium minimum inhibitory concentration (MIC) of tetracycline for *P. fluorescens.* From this MIC, minimum tolerance concentration (MTC) of tetracycline, i.e., 475 µg/20 ml, was determined. This MTC of tetracycline was also added in Kings B throughout the study to make the medium specific for the used strain of *P. fluorescens.* The MIC could not be determined for *Bacillus subtilis* as the strain was found sensitive to all antibiotics. Inhibition zones produced by different antibiotic discs in *B. subtilis*-inoculated plates were measured. The measurements were used to compare the applied strain of *B. subtilis* with the indigenous strains during determination of soil population. This technique is not very accurate for the characterization of *B. subtilis,* but, because of highly sensitive response of *B. subtilis* to the used antibiotics, this technique gives some indication about the applied strain. Seven-day-old culture broth of *P. chlamydosporia* [homogenized suspension with 10^5 colony-forming units (CFU)/ml] and 2 to 3-day-old broths of *B. subtilis* and *P. fluorescens* (10^8 CFU/ml) were used to give root-dip treatment to the seedlings of ornamental plants.

Plant culture and treatments: A field of 30 × 22-m dimension was prepared in which 90 microplots (3 × 2 m) were outlined by 20-cm raised and 20-cm wide margins. Background population of root-knot nematode juveniles (J2) in the field was 17 to 42 J2/kg soil before incorporation of any treatment. No treatment was given to eliminate the background population as it was too low to affect crop growth. Four-week-old seedlings of hollyhock (*Althea rosea* L. cv Indian Spring), petunia (*Petunia hybrida* Vilm. cv Silver Spring), and poppy (*Papaver rhoeas* L. cv Bride) were obtained from the Department of Lands, Gardens and Parks, Aigarh Muslim University. Roots of the seedlings were immersed for 15 minutes in the liquid cultures of *P. chlamydosporia, B. subtilis,* and *P. fluorescens* separately. A similar root-dip treatment with fenamiphos (200 µg/ml) was given for 5 minutes to another group of seedlings. The seedlings without any rinse were planted in microplots (30 microplots for each plant species). Four rows (50 cm apart), each with 10 plants (30 cm apart), were maintained in a microplot. Ten treatments were given to each ornamental species: (i) plant alone, (ii) plant + *P. chlamydosporia,* (iii) plant + *B. subtilis,* (iv) plant + *P. fluorescens,* (v) plant + fenamiphos, (vi) plant + nematode, (vii) plant + nematode + *P. chlamydosporia,* (viii) plant + nematode + *B. subtilis,* (ix) plant + nematode + *P. fluorescens,* and (x) plant + nematode + fenamiphos. Three randomly arranged microplots were maintained for every treatment.

The plants were grown for 4 months (mid November 2000 and 2001 to mid March 2001 and 2002). Number of flowers was recorded weekly from 75-day-old plants until harvest. Microplots were flooded with water before harvesting to facilitate greater root mass recovery. Ten randomly selected plants from each microplot were randomly uprooted, and plant length and number of branches were counted. The root systems were gently rinsed in a slow stream of water, and number of galls and egg masses were counted. To facilitate counting, egg masses were stained by immersing the root systems of infected plants in 0.0095% phloxin B solution for 15 minutes (Daykin and Hussey, 1985).

Soil population densities of M. incognita and biocontrol agents: The population densities of *P. chlamydosporia, B. subtilis, P. fluorescens,* and *M. incognita* were determined monthly in the microplots treated or not treated with the organisms. A soil sample of approximately 1.5 kg was collected from the soil of 10 randomly selected plants in each microplot without uprooting the plants or damaging the root system. One kg soil was used to determine the soil J2 population of *M. incognita* from each microplots. The soil was mixed in 8 liters of water and decanted over a set of 500-, 75-, and 26-µm-pore sieves. Residue from the 26-µm-pore sieve was gently poured over a two layers of tissue paper on a 6-cm coarse sieve. The sieve was placed in a Baermann funnel, and after 12 hours nematode suspension was collected from the funnel and examined using a stereomicroscope.

Soil population densities of biocontrol agents were determined by dilution plate method (Seeley and Van Demark, 1981). Ten grams of soil from each soil sample was mixed in 100 ml double distilled water (DDW) and shaken for 15 minutes. One ml from this suspension was transferred to a test tube containing 9 ml DDW. The process was repeated to achieve dilutions of 10^{-4} and 10^{-6}. Suspension of 10^{-4} dilution (0.25ml/plate) was spread on the potato carrot agar (PCA) in four petri dishes to estimate the colony-forming units (CFU) of *P. chlamydosporia.* The cultures were incubated in a biological oxygen demand (BOD) incubator at 25 ± 2 °C for 6 to 8 days, after which the plates were examined using a colony counter. The fungus was identified on

the basis of morphological characters (Zare et al., 2001).

Population densities of *B. subtilis* and *P. fluorescens* were determined from the 10^{-6} dilution, spreading 0.25 ml suspension on nutrient agar and Kings B supplement plus 475 µg tetracycline/20 ml medium in petri dishes, and incubated in a BOD incubator at 36 ± 2 °C for 2 to 3 days. Four cultures were prepared for each treatment. The cultures were examined using a colony counter. To further confirm identity of the applied strain of *P. fluorescens,* tests for growth, motility, fluorescens, gram stain, oxidase, starch hydrolysis, and gelatine were applied to arbitrarily selected colonies. Tests for inhibition due to the antibiotics, growth, motility, gram stain, oxidase, catalase, nitrate, and glucose acidity were performed to identify the applied strain of *B. subtilis* (Holt et al., 1994). In addition, indigenous populations of *P. fluorescens, B. subtilis,* and *P. chlamydosporia* from untreated plots were compared with the populations of respective microorganisms from inoculated plots.

Colonization of eggs, egg masses, and M. incognita females: To determine the frequency of colonization of egg masses by the biocontrol agents, a total of 100 egg masses and 100 females were excised from the galled roots of 10 plants uprooted from each microplot using sterilized forceps and needles. The egg masses and females were surface sterilized by immersing in 0.1% $HgCl_2$ solution for 1 minute. Thereafter, they were rinsed with DDW repeatedly to remove mercury residues. Ten egg masses or females were placed separately on 2% agar in petri dishes. Three petri dishes were prepared for each treatment. The plates were incubated in a BOD incubator at 25 ± 2 °C for 6 to 8 days (*P. chlamydosporia*) and at 36 ± 2 °C for 2 to 3 days (*B. subtilis* and *P. fluorescens*). After incubation, the plates were examined using a colony counter and percent frequency of infection of egg masses and females by the biocontrol agents was determined. Identity of the microorganisms was confirmed by culturing them on solidified potato carrot agar (PCA), nutrient agar, and Kings B supplement plus 475 µg tetracycline/20 ml medium, and performing morphological and physiological tests described previously. A control set was also maintained.

To determine frequency of colonization of eggs of *M. incognita,* 100 surface-sterilized egg masses were shaken vigorously in 0.5% NaOCl for 2 to 3 minutes to liberate eggs. The suspension was quickly poured over a set of 75- and 26-µm-pore sieves and rinsed with water to remove NaOCl residues. The eggs were sterilized by adding 0.2% $HgCl_2$ solution to an equal volume of egg suspension. After 1 minute, the egg suspension having 0.1% $HgCl_2$ was poured over a sterile 26-µm-pore sieve and rinsed repeatedly with DDW to remove mercury residues. Egg suspensions were standardized to 100 eggs/ml water. The aliquot was spread on PCA, nutrient agar, and Kings B supplement plus 475 µg tetracycline/20 ml medium in petri dishes (0.25 ml/dish) separately. The plates containing PCA and NA or KBS were incubated in a BOD incubator at 25 ± 2 °C for 6 to 8 days and 36 ± 2 °C for 2 to 3 days, respectively. Four cultures were prepared for each treatment. After incubation, the plates were examined using a colony counter and the eggs colonized by *P. chlamydosporia, B. subtilis,* and *P. fluorescens* were counted. Identity of the associated microorganisms was confirmed by performing morphological and(or) physiological tests as described.

Statistical analysis: The experiment was conducted during 2 consecutive years (2000–01 and 2001–02) in different fields but under similar agro-climatic conditions. The mean of various observations taken on 10 plants from a microplot was considered as one replicate. Hence, there were three replicates, as three microplots were maintained for each treatment. The data from the 2 years varied ($P = 0.05$); therefore, they were not pooled but analyzed separately by one- or two-factor analysis of variance (ANOVA). Results, however, describe the experiment conducted during 2001–02 because overall response of the variables to various treatments over the years was similar. Least significance difference (LSD) was calculated at $P = 0.05$ (Dospekhov, 1984). The data on colonization of eggs, egg masses, and females by biocontrol agents, and soil population densities were analyzed by two-way ANOVA, and the Duncan's multiple-range test was applied to identify significant treatments.

Results

Symptoms: Infestation of soil with 2,000 eggs/kg soil resulted in stunted plant growth with pale green foliage of petunia, poppy, and hollyhock. The nematode caused characteristic galls. The galling was severe on all ornamental species tested (Fig. 1). The greatest number of galls was recorded on hollyhock, with 397 galls/root system followed by 191 and 155 galls on petunia and poppy, respectively. Gall formation was suppressed ($P = 0.05$) due to application of biocontrol agents. The greatest suppression in the number of galls per plant was recorded in treatments with fenamiphos on hollyhock (79%), petunia, and poppy (50%); the lowest reduction ($P = 0.05$) occurred due to *B. subtilis* (Fig. 1).

Nematode reproduction and soil population: The nematode reproduced efficiently on the tested plant species, with more than 100 egg masses formed on the root system of each species in the control plots (Fig. 1). The greatest number of egg masses per root system developed on hollyhock, followed by petunia and poppy during the 2 years. The fenamiphos treatment reduced the number of egg masses by 51% to 83%, with the greatest reduction on hollyhock. Application of *P. chlamydosporia* suppressed the egg mass count by 49% (poppy),

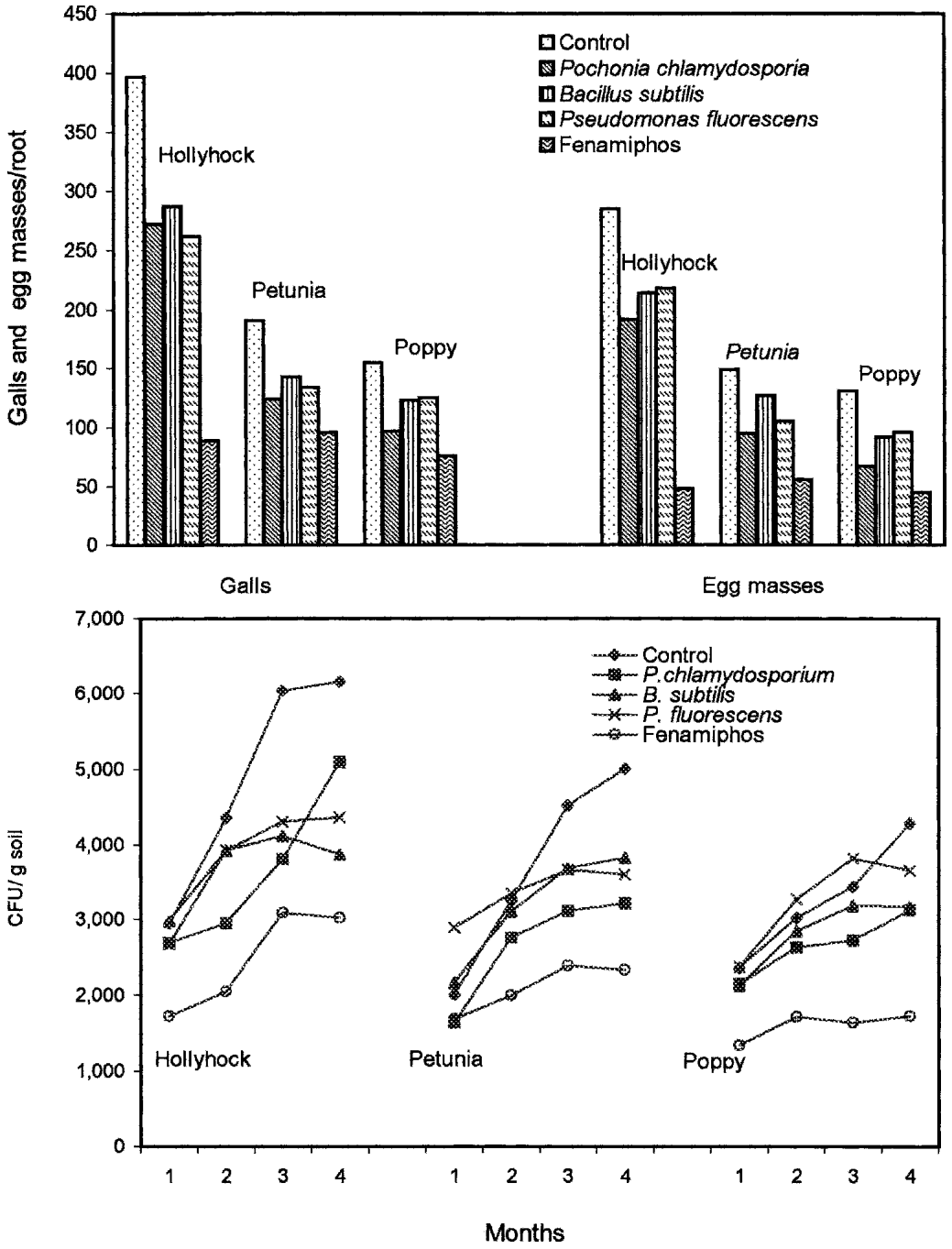

FIG. 1. Effects of root-dip treatment of *Pochonia chlamydosporia, Bacillus subtilis, Pseudomonas fluorescens,* and fenamiphos on gall formation, egg mass production, and monthly soil population densities of *Meloidogyne incognita* on hollyhock, petunia, and poppy.

36% (petunia), and 32% (hollyhock). Treatment with *P. fluorescens* or *B. subtilis* also caused suppressed numbers of egg masses but less so than *P. chlamydosporia* (Fig. 1).

Population densities of *M. incognita* gradually increased over time, being highest at harvest (4 months) on all plants species (Fig. 1). The population also increased over time in the presence of the biocontrol agents or nematicide, but the percentage increase was less than in their absence. The greatest decrease in the population of nematode occurred in treatments with fenamiphos followed by *P. chlamydosporia, B. subtilis,* and *P. fluorescens.*

Soil population densities of biocontrol agents: Population densities of *P. chlamydosporia, B. subtilis,* and *P. fluorescens* in microplots infested or not infested with root-knot nematode gradually increased with time and peaked at 3 months age (Fig. 2). The increase in the CFU of *P. fluorescens* was significant at 2 to 4 months age. Population of *B. subtilis* increased ($P = 0.05$) at 1 month age on petunia and poppy and 3 months age on hollyhock irrespective of nematode infestation com-

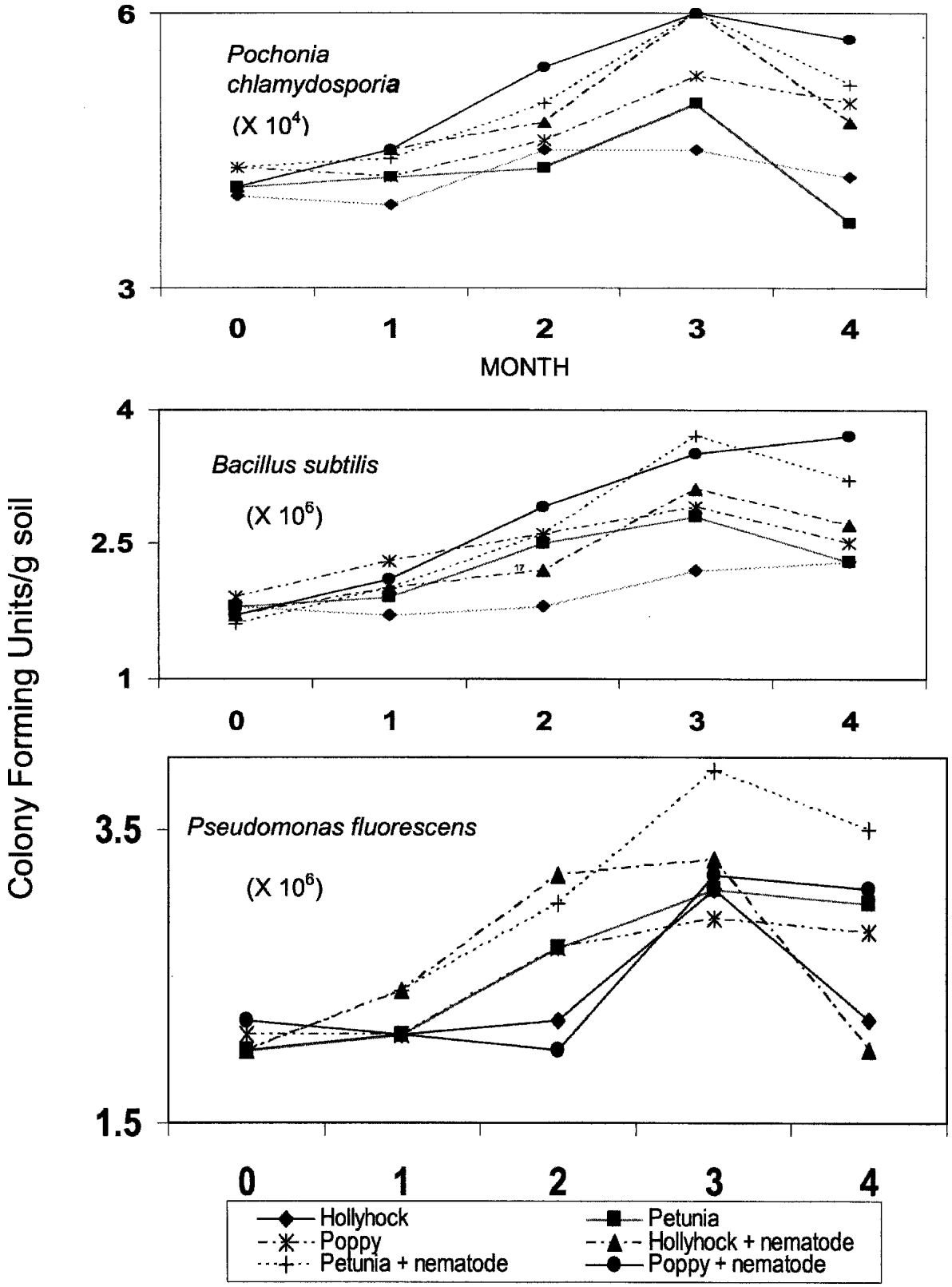

FIG. 2. Monthly rhizosphere population densities of biocontrol agents in the presence and absence of *Meloidogyne incognita* on ornamental plants.

pared to the preplant population densities (Fig. 2). Increase ($P = 0.05$) in the CFU of *P. chlamydosporia* occurred at 2 to 4 months except on poppy. In general, increase in biocontrol agents population densities in the presence of nematode was greater than in their absence ($P = 0.05$) (Fig. 2). Population of *P. chlamydo-*

sporia was greater ($P = 0.05$) in nematode-inoculated plots than uninoculated plots at 2 months age onward (Fig. 2). Difference in the CFU of *B. subtilis* in nematode-infected hollyhock or poppy was significant at 3 and 4 months after planting, and on poppy the decrease in rhizosphere population of the bioagent was significant at 3 months age. The population of *P. fluorescens* on nematode-inoculated petunia and hollyhock was greater than uninoculated plants from 2 and 3 months age, respectively (Fig. 2).

Colonization of eggs, egg masses, and females of M. incognita: Frequency of colonization by biocontrol agents was greater on egg masses than for females, and much lower on eggs (Table 1). The highest frequency of colonization by *P. chlamydosporia* on egg masses was recorded for hollyhock (60%), which was different from petunia or poppy ($P = 0.05$). The frequency of colonization by *B. subtilis* and *P. fluorescens* on egg masses ranged from 27% to 31% and 11% to 13%, respectively. The colonization of eggs by *P. chlamydosporia* was approximately half of the infection of egg masses, ranging from 24% to 29%. Only a few eggs were found to be colonized by *B. subtilis* (0.6% to 1.7%) and *P. fluorescens* (0.2% to 1.0%). The colonization by *P. chlamydosporia* on females was greater than eggs but less than egg masses irrespective of host species (Table 1). The colonization of females by *B. subtilis* and *P. fluorescens* was 14% and 1.0%, respectively.

Plant growth and flowering: Infection by *M. incognita* reduced the plant length and flowering ($P = 0.05$) of the ornamental plants tested in comparision to infested controls (Fig. 3). The percentage decrease of number of flowers per plant was 31% (petunia), 24% (poppy), and 22% (hollyhock). Root-dip treatment with *P. fluorescens* increased ($P = 0.05$) flower production of petunia by 11% to 13% in comparison to noninfested control (Fig. 3).

Application of various treatments suppressed the pathogenic effect of *M. incognita,* leading to an increase in plant growth (Fig. 3). Root-dip treatment with *P. fluorescens* improved the plant length, branches (except hollyhock), and flowers of infected plants compared to the inoculated control ($P = 0.05$). Application of *B. subtilis* increased the flowering in infected poppy and petunia ($P = 0.05$). Root-dip treatment with fenamiphos increased ($P = 0.05$) the plant length and flowers of the ornamental plants. The increase in flowers induced by fenamiphos treatment was 20% and 10% less than that induced by *P. fluorescens.*

TABLE 1. Frequency of colonization (%) by *Pochonia chlamydosporia, Bacillus subtilis,* and *Pseudomonas fluorescens* on eggs, egg masses, and females of *Meloidogyne incognita* collected from the roots of hollyhock, petunia, and poppy.

Biogent		Hollyhock	Petunia	Poppy
P. chlamydosporia	Eggs	24.8 Cc	26.0 Ba	29.3 Ac
	Egg masses	60.0 Aa	54.3 Ba	47.2 Ca
	Females	35.9 Cb	38.7 Bb	41.4 Ab
B. subtilis	Eggs	0.8 Bc	1.7 Ac	0.6 Bc
	Egg masses	30.3 Aa	27.6 Ba	31.2 Aa
	Females	13.9 Ab	14.1 Ab	13.5 Ab
P. flourescens	Eggs	0.2 Bc	1.0 Ab	0.2 Bc
	Egg masses	11.8 Aa	13.3 Aa	10.6 Ba
	Females	1.2 Ab	0.9 Bb	1.2 Ab

Each value is the mean of three replicates; values followed by different upper- and lowercase letters are significantly different within a row or column, respectively, at $P = 0.05$.

Discussion

The tested plant species were susceptible and intolerant to *M. incognita.* Relative tolerance of the three plant species with regard to reduction in plant growth and flower production was in the order of petunia > poppy > hollyhock. Galling and reproduction of *M. incognita* was greatest on hollyhock, followed by petunia and poppy.

Application of *P. fluorescens* promotes plant growth and yield through solubilization of phosphorus (Sakthivel et al., 1986) and(or) production of phytohormones (Glick, 1995). This mechanism may have been responsible for the greater number of flowers on the treated petunia and poppy. Application of *P. fluorescens* through soil (Dubey and Yeole, 1997) or root-dip treatment (Khan and Khan, 2002) may improve plant growth and yield.

Fenamiphos is an efficacious systemic nematicide and its application suppresses root-knot nematode, thus increasing crop yield (Johnson, 1985). Similar effects of fenamiphos treatment were observed on gall formation, egg mass production, and population densities of *M. incognita,* and of growth and flowering of the ornamental species tested. *Bacillus subtilis* and *P. fluorescens* produce nematoxic metabolites that may have been involved in the nematode suppression (Gokte and Swarup, 1988; Khan and Akram, 2000; Siddiqui and Etshamul-Haque, 2001). *Bacillus subtilis* produces the metabolites bacillomycin (Bessen and Michel, 1984; Brannen, 1995), iturin, surfactin (Asaka and Shoda, 1996), and agrocin (Kim et al., 1997). Phlorogucinol (Howell and Stipanovic, 1984), pyrollnintrin (Leyns et al., 1990), and phenazin (Gurusiddaiah et al., 1986) are produced by *P. fluorescens. Pochonia chlamydosporia* is an established biocontrol agent of root-knot nematodes; the fungus parasitizes nematodes and their eggs (Khan et al., 2001; Stirling, 1991). In the present study, nematode pathogenesis was less suppressed by application of *P. fluorescens* than fenamiphos or *P. chlamydosporia,* but overall enhancement in plant growth and flowering was greater with *P. fluorescens.* This may have occurred cumulatively through growth promotion and nematode antagonism. Although fenamiphos and *P. chlamydosporia* provided greater degree of control of root-knot, these treatments did not directly promote plant growth.

Frequency of colonization of egg masses, eggs, and

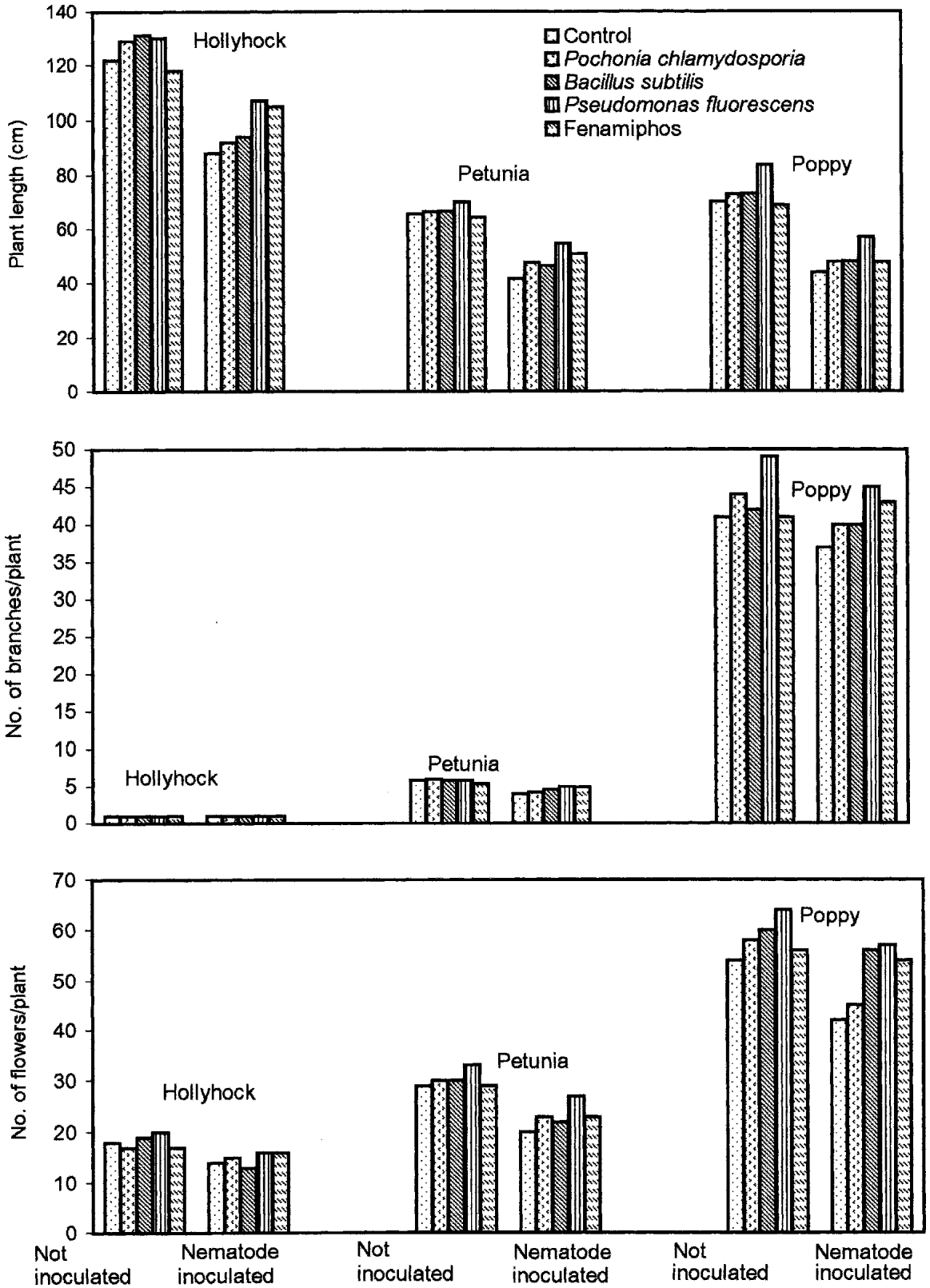

FIG. 3. Effects of root-dip treatment of *Pochonia chlamydosporia, Bacillus subtilis, Pseudomonas fluorescens,* and fenamiphos on plant length, branches, and flower production of hollyhock, petunia, and poppy inoculated with *Meloidogyne incognita.*

M. incognita females by *P. chlamydosporia* was much greater than by *B. subtilis* or *P. fluorescens. Pochonia chlamydosporia* is a parasite of eggs, and the mycellium may invade adult females (Morgan-Jones and Rodríguez-Kábana, 1988). *Bacillus subtilis* is not a parasite of nematodes; however, association with the galled tissue and

egg masses has been reported (Weller, 1988). *Pseudomonas fluorescens* has not been reported to be associated with egg masses, eggs, or females of *Meloidogyne* species. Although frequency of infection by *P. fluorescens* on egg masses was low, this was not so small that it could account for contamination. The gelatinous matrix of the egg mass might have served as a nutritive material for colonization by the bacterium. The matrix of egg mass remains in direct contact of soil containing bacteria. This may have provided the opportunity to *P. fluorescens* or *B. subtilis* to colonize egg mass. The colonization, however, remained confined to surface and the bacteria could not reach inside the egg masses, evidenced by an almost 0% frequency of colonization by *B. subtilis* or *P. fluorescens* on eggs. Adult females of *M. incognita* are well embedded in root tissue and the bacterial cells are non-motile; hence, the chance for contact with the bacterium was remote. As a result, a very low or negligible frequency of colonization of adult females was recorded.

Frequency of colonization on egg masses by *P. chlamydosporia* varied with host species with greatest colonization on petunia. The egg masses on petunia were relatively large, providing greater surface area and substrate for colonization. Because *Bacillus subtilis* or *Pseudomonas fluorescens* are not established colonizers of nematodes, size of egg mass did not influence the frequency of colonization. Greater increase in CFUs of bioagents in the root zone of nematode-infected plants may have been due to leakage of photosynthates from plant roots through exudates (Kerry, 2000). Carbon and several metal ions have been reported to be present in greater concentration in the rhizosphere of plants infected by root-knot or cyst nematodes, which may enhance multiplication of microorganisms (Van Gundy et al., 1977; Yeates et al., 1998). Nematodes and their eggs also may have served as a food source for *P. chlamydosporia, P. fluorescens,* and *B. subtilis.*

The present study has demonstrated that root-knot is an important disease of hollyhock, petunia, and poppy and decreased the production of flowers. Application of fenamiphos or *P. chlamydosporia* decreased the disease severity and reproduction of the nematode, but this did not result in enhanced flower production. Treatment with *P. fluorescens,* however, appeared useful and of practical importance. Application of *P. fluorescens* improved the flowering of nematode-infected and uninfected ornamental plants greater than nematicide and to a level that could be exploited commercially.

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