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Fish emulsion as a food base for rhizobacteria promoting growth of radish (*Raphanus sativus* L. var. *sativus*) in a sandy soil

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Abstract

Commercial fish emulsion was evaluated as a plant growth medium and as a nutrient base to enhance radish (*Raphanus sativus* L. var. *sativus*) growth by bacterial and actinomycete isolates. Six bacterial isolates including three actinomycetes were selected from a screening of 54 bacteria (including 23 actinomycetes) based on their ability to produce plant growth regulators (PGRs) and to colonize radish roots. These isolates were tested in the presence and absence of autoclaved or non-autoclaved fish emulsion or inorganic fertilizers. The nutrient contents and types and levels of PGRs in tissues of treated plants were assayed to determine the basis of growth promotion. Fish emulsion was found to support plant growth in a sandy soil as effectively as an applied inorganic fertilizer. The plant growth promotion by bacterial and actinomycete isolates was most pronounced in the presence of autoclaved or non-autoclaved fish emulsion than in the presence of the inorganic fertilizers. The bacterial and actinomycete isolates were capable of producing auxins, gibberellins and cytokinins and appeared to use fish emulsion as a source of nutrients and precursors for PGRs. PGR levels *in planta* following combined treatments of the bacterial and actinomycete isolates and fish emulsion were found to be significantly enhanced over other treatments. The effect of fish emulsion appears to be more related to its role as a nutrient base for the bacterial and actinomycete isolates rather than to the increased activity of the general microflora of treated soil. This is the first report of fish emulsion as a nutrient base for plant growth promoting rhizobacteria. These results also indicate that the successful treatment can be effective and economical for horticultural production in sandy soils such as those found in the United Arab Emirates where fish emulsion is already in use as a substitute or supplement for inorganic fertilizer.

Introduction

Many current farming practices exist where increased plant yield and productivity are obtained by amending the soil with a variety of organic amendments such as animal and/or plant manures (Bulluck and Ristaino, 2002; Li et al., 2000; Seesahai and Ferguson, 1998). Worldwide, fish-meal powder (Blatt and McRae, 1998; Ndiaye et al., 2000; Sadiq and Hussain, 1993) and soluble fish emulsion (Aung and Flick, 1980; Cheng, 1987; Emino, 1981) have been used as fertilizers, either singly or in combination with other amendments to improve yields of many greenhouse and field crops. In the United Arab Emirates (UAE), farmers currently use organic fertilizers such as fish emulsion, fish meal powder and seaweed ex-

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tract to replace or to supplement inorganic chemical fertilizers.

Fish emulsion and soluble fish meal are traded internationally and are the condensed byproducts of the seafood industry (Soares et al., 1973). In view of their recognized value as fertilizer or as a component in commercial fertilizer formulations, it is desirable to have more definitive information on their effect on plant growth and development in order to document their claimed beneficial effects and uses on plants. There is, however, no published information on the relative effectiveness of the nutrients in fish emulsion on the growth of specific crops in the presence of micro-organisms comprising plant growth promoting rhizobacteria (PGPR).

PGPR are a class of beneficial free-living bacteria inhabiting the soil ecosystem (Kloepper et al., 1989; Whipps, 2001) that are capable of stimulating plant growth either indirectly or directly. Indirect mechanisms of growth promotion include the production of iron-sequestering siderophores (preventing iron acquisition by deleterious microorganisms) (O'Sullivan and O'Gara, 1992), production of compounds that may have antimicrobial or antifungal properties and serve to protect plants from soil phytopathogens (Kloepper et al., 1989; Whipps, 1997). Direct mechanisms may involve the fixation of atmospheric nitrogen that may be used by the plant (Noel et al., 1996; Tien et al., 1979), the facilitation of the uptake of minerals such as phosphate into the plant (Kloepper et al., 1988) or the production of plant growth regulators (PGRs) such as auxins, gibberellins and cytokinins which in very low quantities stimulate plant growth (Kloepper et al., 1989).

The overall aim of this study was to determine whether the growth promotion of radish from fish emulsion was mainly a response to the stimulatory activity of resident micro-organisms in the commercial emulsion or indigenous soil micro-organisms and whether the emulsion could enhance the plant growth promotion effect of selected micro-organisms isolated from radish rhizosphere.

In this investigation, we selected bacteria and actinomycetes for their response to fish emulsion based on their ability to produce PGRs and to effectively colonize radish roots. This selection resulted in the reduction of the total number of microorganisms used for the final glasshouse trial from 54 bacterial isolates (including 23 actinomycetes) to six isolates including three actinomycetes. In the glasshouse trial, various combinations of treatments were tested to determine the potential of fish emulsion to substitute for inorganic fertilizer and to enhance the plant growth promoting activity of the selected microorganisms. To determine the basis of plant growth promotion by the selected bacterial and actinomycete isolates in the presence or absence of fish emulsion, the microbial activity of amended soil as well as plant nutrient levels and endogenous PGRs were assayed.

Materials and methods

Isolation of bacteria and actinomycetes from radish rhizosphere soil

Field soil following a red radish crop (*Raphanus sativus* L. var. *sativus*) cv. Champion (Petoseed Company, Inc., Saticoy, CA, USA) was collected in January (winter) 2000 from a farm located at Al-Ain city, 140 km east of Abu-Dhabi, United Arab Emirates (UAE). The soil was a light yellowish brown sandy soil with a pH of 7.4 (in 0.01 M $CaCl_2$). The 20 cm diameter free-draining pots (Smith and Nephew, Vic, Australia) were filled with 6 kg of air-dried soil. The soil was amended with 200 mL pot^{-1} of Yates fish emulsion (Arthur Yates & Co Limited, Milperra, NSW, Australia) at the manufacturer's recommended rate (2 mL fish emulsion l^{-1} water) every 10 days for 2 months before sowing radish seeds in the amended soil. In between the fish emulsion applications, the soil was watered to container capacity every 4 days. There were 5 replicate pots with 5 seeds pot^{-1}. After sowing, two further applications of fish emulsion (at 0 and 10 days) were made. At day 20, radish rhizosphere samples were collected by removing the roots and shaking the adhering soil into plastic bags. In the laboratory, each rhizosphere soil sample (5 samples) were passed through a 1 cm mesh sieve and stored in sealable plastic bags at 5 °C for less than 1 week prior to processing.

For the isolation of bacteria and actinomycetes, three 10 g replicates of each rhizosphere soil sample were dispensed into 100 mL of sterile 1 g l^{-1} agar (Gibco BRL, Paisley, Scotland) solution in deionized water containing 20 g glass beads (3 mm diameter). The soil suspension was placed in an ultra-sonicator (Virsonic 60, the Virtis Company, Inc., Gardiner, NY, USA) at a frequency of 55 000 cycles sec^{-1} for 20 s, and then shaken on a rotary shaker (Model G76, New Brunswick Scientific, Edison, NJ, USA) at 250 rpm for 30 min at 28 °C. Ten-fold dilutions

(10^{-2} – 10^{-5}) were made in sterile deionized water and 0.2 mL aliquots were spread with a sterile glass rod over the surface of modified Hussein's fish meal extract agar (El-Tarabily et al., 1997) in sterile plastic, 9 cm diameter Petri-plates. The modification involved the incorporation into the medium of commercial fish emulsion instead of fish meal powder. Cooled (45 °C) sterile Hussein's fish emulsion (HFE) agar was amended with cycloheximide (Sigma Chemical Company, St Louis, MO, USA) (50 mg l^{-1}) and nystatin (Sigma) (50 mg l^{-1}) immediately prior to pouring plates. Ten plates were used per dilution and dried in a laminar flow-cabinet for 15 min before incubation at 28 ± 2 °C in the dark for 7 days.

Bacterial colonies were transferred onto nutrient agar plates (BBL, Becton Dickinson, Cockeysville, MD, USA), whilst, actinomycete colonies were transferred onto oatmeal agar plates supplemented with 1 g l^{-1}yeast extract. The bacterial cells or the hyphae and spores of actinomycetes from five plates were removed from the culture surface, suspended and stored in 10% glycerol (cryoprotectant) at –20 °C (Wellington and Williams, 1978).

To determine if there were resident micro-organisms in the commercial fish emulsion (four replicates), ten-fold dilutions (10^{-1} – 10^{-4}) were made in sterile deionized water and 0.2 ml aliquots were spread with a sterile glass rod over the surface of HFE agar in sterile plastic, 9 cm diameter Petri-plates. Five plates were used per dilution and dried in a laminar flow-cabinet for 15 min before incubation at 28 ± 2 °C in the dark for 7 days. Micro-organisms from the isolation plates were transferred to the purification media as described above.

Preliminary screening for PGRs production by bacteria and actinomycetes

The aim of this experiment was to screen the 54 rhizosphere bacterial isolates (including 23 actinomycetes) for their ability to produce PGRs, including auxins, gibberellins and cytokinins, in liquid HFE. Erlenmeyer flasks (250 mL), each containing 50 mL of liquid HFE were inoculated with 5 mL of each of the isolated bacteria or actinomycetes, covered with aluminum foil and incubated on a rotary shaker at 250 rpm at 28 ± 2 °C in the dark for 10 days, to avoid photo-oxidation of the PGRs.

Extraction of PGRs

After 10 days, the broth containing bacterial or actinomycete cultures (200 ml) was centrifuged at 7500 *g* for 30 min. The supernatant was reduced to 50 mL by evaporation under vacuum and the supernatant fluid then filtered through 0.22 μm membrane filters (Millipore, Australia Pty Limited, Australia) to remove cell debris. The concentrated filter-sterilized cell-free culture broth was acidified with HCl to pH 3 and was shaken three times with ethyl acetate (HPLC grade) (Sigma) at 30 min intervals as described by Tien et al. (1979). The combined ethyl-acetate fractions were collected for the identification of auxins and gibberellins. The aqueous fraction that was obtained after the extraction with ethyl acetate was adjusted to pH 7 with NaOH and was shaken three times with water-saturated n-butanol (Sigma) at 30 min intervals and the n-butanol fractions were collected for the identification of cytokinins (Tien et al., 1979). Both ethyl acetate and n-butanol fractions were evaporated to dryness in a rotary evaporator under vacuum at a temperature of 40 °C and the residues were re-dissolved in 1 ml of absolute methanol (HPLC grade) (Sigma). The methanol extracts were stored in the dark at –20 °C until further use for thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Sterile HFE broth (control) was also extracted and examined for PGRs. There were four replicates for each isolate.

Identification of PGRs

Co-chromatography with authentic PGR compounds by TLC, specific color reactions with chromogenic reagents and bioassays were used to establish PGR identity. The chromatograms were run on 0.50 mm thick preparative silica gel plates (Sigma). A freshly prepared solvent consisting of chloroform: ethyl-acetate: formic acid (50:40:10) (v/v) was used to separate auxins and gibberellins in ethyl acetate fractions and n-butanol: acetic acid: water (12:3:5) (v/v) was used to separate cytokinins in n-butanol fractions (Tien et al., 1979). Auxins were detected on TLC plates by spraying the chromatogram with Ehrlich reagent (Bentley, 1962) and the color was allowed to develop for 1 h at 30 °C. Gibberellins were detected by spraying another chromatogram with ethanolic sulfuric acid (90:10) (v/v) and heating to induce fluorescence of the compounds in ultraviolet light (MacMilan and Suter, 1963). Cytokinins were detected by observing the chromatogram under 245 nm UV light and by spraying the chromatogram with a 1% $AgNO_3$ solution in 0.65 *M* NH_4OH and heating at 105 °C as described by Surico et al. (1985). The *Rf* values were calculated for authentic compounds and samples. By comparing the migration distance and the quantity (spot size) for each of the potential PGRs produced by all isolates on the TLC plates with authentic compounds (Sigma), the most powerful PGR producing 14 bacterial isolates (including 6 actinomycetes) were selected for biological assays. The most common PGRs produced in relatively large amounts by the 14 selected isolates were indole-3-acetic acid (IAA), indole-3-pyruvic acid (IPYA), gibberellic acid (GA_3), isopentenyl adenine (IPa), isopentenyl adenoside (IPA) and zeatin (Z). There were four replicates for each isolate.

Bioassay of PGRs

To confirm the presence of PGRs, a biological assay was conducted for all 14 isolates. TLC chromatograms not treated with chromogenic reagents were dried for 7 days to remove solvents and cut transversely into 10 equal sections representing the sequence of R_f values 0.1 to 1.0. These sections were then eluted separately for bioassays. The spot on the plate at the R_f of IAA and IPYA were extracted with methanol and tested for effects on oat coleoptile segment elongation at 25 °C in the dark by the method of Bentley (1962). GA_3 was assayed by the method of Frankland and Wareing (1960) with lettuce hypocotyls. Cytokinins were detected by effects of methanol extracts on chlorophyll retention in oat leaves (Wheeler, 1972). The method described by Strain et al. (1971) was used to determine chlorophyll content. Each bioassay was repeated four times with three replicates in each.

Root colonization and rhizosphere competence assays

Based on the bioassay results, only 12 bacterial isolates (including 5 actinomycetes) were selected to test their ability to colonize radish roots. A preliminary indicator root colonization plate assay (Kortemaa et al., 1994) was carried out *in vitro* to rapidly indicate whether or not the root exudates of radish, acting as the sole carbon source, would support the growth of each isolate. If this was shown, a rhizosphere competence assay using the non-sterile sand tube method described by Ahmad and Baker (1987) was conducted using rifampicin resistant mutants as described by Misaghi (1990). The experiments were repeated four times with three replicates in each.

Identification of bacteria and actinomycete isolates to species level

On the basis of the results obtained from the PGR bioassays, the root colonization plate assay and the rhizosphere competence assay, only six bacterial isolates (including three actinomycetes) were selected for the glasshouse trial.

Gram-negative and -positive bacterial isolates were identified using standard methods including Gram stain and general morphology, spore formation, colony morphology, pigmentation, physiological and biochemical tests as described by Palleroni (1984) and Sneath (1986), respectively. The API 20E diagnostic strips (bioMerieux sa, Marcy l'Etoile, France) and the API 20B (API System SA, La Balme Les Grottes, Montalieu, Vercieu, France) were also used in the identification of Gram-negative and -positive bacteria, respectively. These isolates were identified as *Pseudomonas fluorescens* Trevisan (isolate # 4), *Pseudomonas putida* Trevisan (isolate # 17) and *Bacillus pumilus* Meyer and Gottheil (isolate # 21).

Identification of the three actinomycete isolates to species level was based on morphological, cultural, biochemical, physiological and chemotaxonomical characteristics as described by Locci (1989). These were identified as *Streptomyces griseoflavus* Krainsky (isolate # 7), *Streptomyces rimosus Sobin* et al. (isolate # 9) and *Streptomyces diastaticus* Krainsky (isolate # 12).

Glasshouse trial

Bacteria and actinomycetes inoculum production

The inoculum for each isolate was prepared by placing 50 g of moist wheat bran into 500 mL Erlenmeyer flasks and autoclaving at 121 °C for 20 min on three consecutive days as described by El-Tarabily et al. (2000). The substrate was then aseptically inoculated with a 30 mL spore/cell suspension (10% glycerol) of each isolate and incubated at 28 ± 2 °C in the dark for 2 weeks. The flasks were routinely shaken to ensure uniformity of colonization. Prior to use, small amounts of the colonized and control wheat bran were suspended in 25 mL of sterile distilled water. Aliquots (0.3 mL) of these suspensions were spread onto nutrient agar and oatmeal agar plates to confirm the presence or absence of the bacterial and actinomycete isolates, respectively.

Soil infestation

Wheat bran colonized with each bacterium or actinomycete isolate was thoroughly dispersed through the air-dried field soil by mixing in a cement mixer (0.1 g colonized wheat bran inoculum g^{-1} air-dried non-sterile soil). Twenty-cm diameter free-draining pots were filled with 6 kg of infested soil, left for 2 weeks before sowing the seeds and were watered twice a week. In total, there were 17 treatment combinations: (1) control (moist soil with radish plants only), (2) + fish emulsion, (3) + autoclaved fish emulsion, (4) + inorganic fertilizer applied as a foliar spray, (5) + inorganic fertilizer applied as a soil drench, (6) + bacterial mixture + inorganic fertilizer applied as a soil drench, (7) + actinomycete mixture + inorganic fertilizer applied as a soil drench, (8) + bacterial mixture + actinomycete mixture + inorganic fertilizer applied as a soil drench, (9) + bacterial mixture + fish emulsion, (10) + bacterial mixture + autoclaved fish emulsion, (11) + bacterial mixture, (12) + actinomycete mixture + fish emulsion, (13) + actinomycete mixture + autoclaved fish emulsion, (14) + actinomycete mixture, (15) + bacterial mixture + actinomycete mixture + fish emulsion, (16) + bacterial mixture + actinomycete mixture + autoclaved fish emulsion and (17) + bacterial mixture + actinomycete mixture. In treatments 1, 2, 3, 4 and 5 autoclaved non-infested wheat bran was used.

The inorganic fertilizer used was an all-purpose soluble chemical fertilizer (Thrive®, Arthur Yates & Co Limited, Milperra, NSW, Australia) (NPK 27:5.5:9). The chemical analysis (%) of the fertilizer was as follows: N as NO_3^-3, N as NH_4 2.6, N as NH_2CONH_2 21.4, P as water soluble 5.5, K as KNO_3 9, Mg as $MgSO_4$ 0.15, S as SO_4 0.22, Cu as $CuSO_4$ 0.005, Zn as $ZnSO_4$ 0.02, B as $Na_2B_4O_7$ 0.005, Mn as $MnSO_4$ 0.04, Fe as chelated Fe 0.18 and Mo as Na_2MoO_4 0.002. The applications of 200 ml pot^{-1} (1.7 g l^{-1} water) were made once every 10 days.

The commercial fish emulsion used was made from pure fish residue (NPK 4.5:1.8:1.2) with the following chemical analysis (%): N as NO_3^-0.005, N as NH_4 1.58, N as NH_2CONH_2 2.9, P as water soluble 1.84, K as KNO_3 1.24, Mg 0.03, S 1.34, Ca 1.05, Na 0.155, Cu 0.00005, Zn 0.0003, B 0.0001, Mn 0.0002, Fe 0.003, Cl 1.89, Mo 0.00009 and C 25.2. The applications of 200 mL pot^{-1} (2 mL fish emulsion l^{-1} water) were made once every 10 days.

Radish seeds were surface-sterilized in 70% ethyl alcohol for 1 min followed by immersion in 0.5% NaOCl for 2 min. Surface-sterilized seeds were then washed seven times with sterile distilled water, and pre-germinated on moist filter paper at 28 ± 2 °C in the dark for 24 h, to obtain uniform seedlings. The germinated seeds (10 pot^{-1}) were sown into the pots containing the required soil treatments to a depth of 5 mm in each pot and when emergence was complete (ca. 4 days) the seedlings were thinned to five pot^{-1}. Each treatment was replicated six times with five plants $replicate^{-1}$, in a fully randomized block design. The pots were placed in an evaporatively cooled glasshouse and maintained at 23°C ± 3 °C. The pots were watered daily to container capacity and fertilized at sowing and every 10 days with the fish emulsion (treatments 2, 9, 12 and 15), with autoclaved fish emulsion (treatments 3, 10, 13 and 16), with inorganic fertilizer applied as foliar spray for treatment 4 or with inorganic fertilizer applied as a soil drench (treatments 5, 6, 7 and 8) at the manufacturer's recommended rate. Whilst treatments (1, 11, 14 and 17) received only water. Plant growth was monitored by recording the fresh and dry weight of roots and shoots, length of roots and shoots and width of roots when they were of marketable size at the time of harvest (8 weeks after sowing).

Estimation of the total microbial activity

In order to compare the effect of fish emulsion or inorganic fertilizer on the soil microbial activity, only three treatments were chosen. These treatments were the non-amended soil (treatment 1), soil amended with autoclaved fish emulsion (treatment 3) and soil amended with inorganic fertilizer applied as a soil drench (treatment 5). The microbial activity of the freshly sampled rhizosphere soils was measured at sowing and after 4 and 8 weeks by the fluorescein diacetate (FDA) hydrolysis technique as described by Schnurer and Rosswall (1982). The experiment was repeated two times with ten replicates in each and the results were converted to μg hydrolyzed FDA g^{-1} dry soil.

Plant nutrient analyses

Comparisons were made between the plant growth responses in soil treated with autoclaved fish emulsion (treatment 3), inorganic fertilizer applied as a soil drench (treatment 5), inorganic fertilizer applied as a soil drench combined with bacterial and actinomycete mixture (treatment 8) and autoclaved fish emulsion combined with bacterial and actinomycete mixture (treatment 16) to determine the overall effect on nutrients and PGR levels in relation to the enhanced plant growth. Because treatment 16 gave the best yield in the glasshouse trial even in comparison to the treatment which included the application of bacterial mixture combined with autoclaved fish emulsion (treatment 10), or the treatment which included the application of actinomycete mixture combined with autoclaved fish emulsion (treatment 13), it was considered to be the most appropriate treatment for nutrient and PGRs analysis. Nutrients and PGRs analysis of materials from treatment 8 was included because the treatment gave a better yield than treatment 6 (bacterial mixture combined with inorganic fertilizer) or treatment 7 (actinomycete mixture combined with inorganic fertilizer). The data from treatment 8 lent itself for the analysis of the microbial role in nutrient supply. Treatments 3 and 5 served as controls.

After harvest, radish roots and the youngest fully opened leaves were selected at random, washed in deionized water, sliced into small pieces and oven dried overnight at 70 °C. The roots and shoots were then analyzed for mineral nutrients as follows. For the measurement of N, finely ground plant material was combusted at 950 °C in oxygen using a LECO FP-428 Nitrogen Analyzer. The released N from the sample was measured as it passed through a thermal conductivity cell (Sweeney and Rexroad, 1987). For Cu, Zn, Mn, Ca, mg, Na, Fe, K, P and S, plant material was digested in a 9:1 mixture of nitric and perchloric acid and measured by inductively coupled plasma atomic emission spectrometry (McQuaker et al., 1979). For the measurement of Cl and NO_3^-, plant material was extracted in deionized water and the Cl and NO_3^--N were measured simultaneously using a Lachat Flow Injection Analyzer. The NO_3^- was reduced to NO_2^- through a copperised cadmium column and the nitrate was measured colorimetrically at 520 nm. The concentration of Cl was measured colorimetrically at 480 nm (Zall et al., 1956). For the measurement of B, plant material was dry-ashed and the ash was extracted with dilute acid. B was then measured colorimetrically with Azomethine H (Gaines and Mitchell, 1979).

Extraction of endogenous PGRs from radish roots and shoots

The extraction of endogenous PGRs from the roots or the apical parts of the shoots including the terminal bud and the first youngest fully opened leaves was carried-out as described by Guinn et al. (1986) and Shindy and Smith (1975). Briefly, the tissues were quickly frozen at –85 °C, and ground in cold 80% extracting solvent methanol-butylated hydroxytoluene-sodium ascorbate. The macerate was then transferred to a flask with fresh extracting solvent and the volume was adjusted to 20 mL and filtered. The filtrate was evaporated to the aqueous phase in a rotary flash evaporator at 35 °C. The aqueous phase was then adjusted to pH 8 with K_2HPO_4 and the sample was partitioned three times with equal volumes of washed ethyl acetate-butylated hydroxytoluene. The dried basic ethyl acetate fraction was kept for cytokinin determination, whilst the aqueous phase was adjusted to pH 2.8 with H_3PO_4 (Sigma). The acidified solution was passed through C_{18} cartridge (Waters Associates, Sep-Pak) to trap auxins and gibberellins. Auxins and gibberellins were then eluted with NH_4OH (Sigma) and the pH quickly adjusted to 2.8 with H_3PO_4. The aqueous eluted phase was partitioned three times, each with 10 mL portion of washed diethyl ether-butylated hydroxytoluene. The ether was evaporated by rotary flash evaporation at 40 °C, the residue was immediately dissolved in methanol and the sample was ready for injection into the HPLC for determination of the acidic PGRs.

For cytokinin extraction, the dried basic ethyl acetate fraction was dissolved in 80% methanol-butylated hydroxytoluene-sodium ascorbate. The solvent was evaporated under vacuum leaving an aqueous phase that was adjusted to pH 2.8 with H_3PO_4 and partitioned three times with washed ethyl acetate-butylated hydroxytoluene. The aqueous phase was adjusted to pH 5.5 with K_2HPO_4 and partitioned three times with water-saturated n-butanol-butylated hydroxytoluene. Cytokinins were trapped on a SepPak C_{18} cartridge, from which they were eluted by 80% methanol. The methanol was evaporated to dryness, and the sample was dissolved in an HPLC solvent phase as described by Machàckovà et al. (1993) and the sample was then ready for injection into the HPLC.

Table 1. Effect of application of fish emulsion or inorganic fertilizer to soil with or without bacteria and/or actinomycetes on growth characteristics of radish grown in an evaporatively cooled glasshouse maintained at 23 ± 3 °C

Treatments*	Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)	Root width (cm)
(1) Control (moist soil with radish plants only)	0.20 *a*	0.05 *a*	6.10 *a*	1.03 *a*	0.45 *a*
(3) + Autoclaved fish emulsion	1.08 *e*	0.65 *e*	19.57 *e*	5.27 *e*	2.18 *e*
(5) + Inorganic fertilizer	1.06 *e*	0.62 *e*	19.31 *e*	5.30 *e*	2.24 *e*
(6) + Bacterial mixture + inorganic fertilizer	1.45 *f*	0.75 *f*	20.84 *f*	6.21 *f*	2.77 *f*
(7) + Actinomycete mixture + inorganic fertilizer	1.57 *g*	0.81 *g*	21.47 *g*	6.52 *g*	2.88 *g*
(8) + Bacterial + actinomycete mixture + inorganic fertilizer	1.83 *h*	1.07 *h*	27.18 *h*	7.67 *h*	3.12 *h*
(10) + Bacterial mixture + autoclaved fish emulsion	2.05 *i*	1.18 *i*	28.62 *i*	9.71 *i*	3.58 *i*
(11) + Bacterial mixture	0.54 *b*	0.24 *b*	9.63 *b*	2.11 *b*	1.12 *b*
(13) + Actinomycete mixture + autoclaved fish emulsion	2.09 *j*	1.38 *j*	30.92 *j*	10.45 *j*	3.73 *j*
(14) + Actinomycete mixture	0.60 *c*	0.34 *c*	11.65 *c*	3.26 *c*	1.22 *c*
(16) + Bacterial + actinomycete mixture + autoclaved fish emulsion	2.37 *k*	1.61 *k*	35.26 *k*	11.04 *k*	4.00 *k*
(17) + Bacterial + actinomycete mixture	0.80 *d*	0.44 *d*	14.15 *d*	4.24 *d*	1.45 *d*

*The radish were grown in soils amended with or without fish emulsion, inorganic fertilizer, bacteria and/or actinomycetes. Plants were harvested after 8 wk. Values are means of six replicate pots (5 plants pot^{-1}). The values with the same letter within a column are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test. No significant differences were obtained between the treatments that included non autoclaved or autoclaved fish emulsion singly or in combination with bacteria and/or actinomycetes and accordingly the data of autoclaved fish emulsion are presented.

HPLC analysis of PGRs in radish roots, shoots and in culture filtrates of bacteria and actinomycete isolates

For the measurement of PGR levels in radish roots and shoots and in the individual extracts of the six bacterial isolates (including three actinomycetes), the parameters described by Tien et al.(1979) were applied. The bacterial isolates were grown in HFE broth and in a standard medium (SM) (Katznelson and Cole, 1965). The actinomycete isolates were also grown in HFE broth and in a modified SM (Katznelson and Cole, 1965). Inoculation of the media and the extraction of PGRs in SM were as described above for HFE broth. Sterile HFE broth and SM broth (controls) were also extracted and examined for PGRs.

HPLC chromatograms were produced by injecting 10 μL of the methanol dissolved sample onto a 10-μm reverse phase column (Waters Associates μBondapak C_{18}, 4 mm × 30 cm) in a Waters Associates liquid chromatography equipped with a differential ultraviolet detector. Two isocratic solvent systems were used to separate auxins, gibberellins and cytokinins as described by Tien et al. (1979). The concentrations of PGRs were obtained by comparing their respective peak areas in the unknown sample with their corresponding areas obtained with the authentic samples (Sigma) of a known concentration.

Statistical analysis

The treatments were arranged in a randomized complete block design for all experiments. To evaluate the effect of different amendments on plant productivity in the glasshouse trial and on the levels of plant nutrients and PGRs, data were subjected to one way analysis of variance (ANOVA) and significant differences between means were compared by Fisher's Protected LSD Test at $P = 0.05$. Percentage data of root colonization were arcsine transformed before analysis of variance was carried out. This transformation improved normality of the distribution of the data and made group variances homogenous. Superanova® (Abacus Concepts, Inc., Berkeley, CA, USA) was used for all analyses.

Results

Preliminary screening for PGRs production by bacteria and actinomycetes

In the commercial fish emulsion, only bacteria were isolated (cfu = 22 × 10^2 mL^{-1}) (SE = 0.76). Fifty-four bacterial isolates (including 23 actinomycetes) were isolated from the radish rhizosphere. Out of the 54 isolates, only 14 isolates (including 6 actinomycetes) were selected based on their ability to produce PGRs in HFE broth. The rest of the isolates were either non-producers of PGRs or produced only a very small amount of PGRs as detected by the spot size on the TLC and consequently were not included in the subsequent studies.

TLC analysis of culture extracts of the 14 isolates showed clear spots at the *Rf* corresponding to the authentic *Rf* of IAA, IPYA, GA_3, IPa, IPA or Z when the chromatograms were treated with the chromogenic reagents. In addition to the identified PGRs, several other auxin, gibberellin and cytokinin-like compounds were detected on the TLC plates at different *Rf* values, but their identities were not determined due to the lack of authentic compounds and consequently no further bioassays of those compounds was carried out. No PGRs were detected on TLC plates with sterile HFE broth.

In the bioassay experiments, substances extracted from bacterial and actinomycete cultures grown in HFE broth and corresponding in position to the authentics IAA, IPYA and GA_3 *Rf* values, produced significant ($P < 0.05$) elongation in the bioassays with oat coleoptiles (IAA and IPYA) and lettuce hypocotyls (GA_3). A significant ($P < 0.05$) increase in the chlorophyll concentrations of the oat leaves was produced by substances extracted from both bacterial and actinomycete isolates with *Rf* values corresponding to authentic IPa, IPA and Z. The extract from one bacterial culture (isolate # 15) and an actinomycete culture (isolate # 19) showed only weak biological activity in the bioassays for IAA and GA_3, respectively and accordingly these isolates were not included in further studies.

Root colonization plate assay

Two of the seven bacterial isolates and two of the five actinomycete isolates failed to colonize the roots in the root colonization plate assay 7 days after emergence and accordingly were not included in the rhizosphere competence assay. *P. fluorescens* (isolate # 4) and *P. putida* (isolate # 17) colonized 95% of roots by 4 days after radicle emergence, whilst *B. pumilus* (isolate # 21) colonized 90% of roots after 5 days. Bacterial isolates # 3 and # 11 colonized 60% and 55% of the roots after 7 days, respectively. *S. rimosus* (isolate # 9) and *S. griseoflavus* (isolate # 7) colonized 95% of the roots by 4 days, whilst *S. diastaticus* (isolate # 12) colonized 90% of roots after 6 days.

Rhizosphere competence assay

Roots and soil particles attached to roots of 14-day-old radish seedlings were colonized to different degrees by the bacterial and actinomycete isolates. The frequency of colonization was significantly ($P < 0.05$) greater in the proximal 2 cm of root and soil nearest to the seed. Colonization frequency of the root segments and the rhizosphere soil was greater in plants treated with *P. fluorescens* and *S. rimosus*, followed by *S. griseoflavus, P. putida*, *B. pumilus* and *S. diastaticus*. Bacterial isolates # 3 and 11 were not included in subsequent studies due to their poor root and rhizosphere colonization abilities.

After 14 days, *P. fluorescens*, *P. putida*, *S. griseoflavus* and *S. rimosus* significantly ($P < 0.05$) colonized roots and associated soil particles down to 10 cm from the stem base. *B. pumilus* and *S. diastaticus* significantly ($P < 0.05$) colonized down to 8 cm of the roots and associated soil particles to varying degrees.

Glasshouse trial

The application of non-autoclaved or autoclaved fish emulsion combined with the bacterial mixture (treatments 9 and 10, respectively) or actinomycete mixture (treatments 12 and 13, respectively) or the combination of both mixtures of bacteria and actinomycetes (treatments 15 and 16, respectively) enhanced the growth and development of radish in the glasshouse experiment (Table 1). Responses to treatment 16 were the best among the treatments attempted. In these treatments there were significant ($P < 0.05$) increases in fresh and dry weight of roots and shoots, root and shoot lengths and root widths compared to the treatments which included the application of non-autoclaved (treatment 2) or autoclaved fish emulsion (treatment 3) without the addition of bacterial and/or actinomycete isolates, or the treatments which did not include fish emulsion (treatments 1, 4, 5, 6, 7, 8, 11, 14 or 17) (Table 1). There was a significant ($P < 0.05$) increase in the root and shoot growth characteristics of radish plants grown in soil amended with the fish emulsion combined with the mixture of bacteria and actinomycetes (treatments 15 and 16) compared to the treatments which included bacterial or actinomycete mixture with the autoclaved fish emulsion (treatments 10 and 13, respectively) (Table 1) or with the non-autoclaved fish emulsion (treatments 9 and 12, respectively).

Table 2. The effect of soil amendments with autoclaved fish emulsion, inorganic fertilizer applied as a soil drench, autoclaved fish emulsion or inorganic fertilizer in combination with bacteria and actinomycetes on the nutrient levels in the roots of radish plants grown in an evaporatively cooled glasshouse maintained at 23 ± 3 °C

Nutrients level	Treatment (3)	Treatment (5)	Treatment (8)	Treatment (16)
Nitrogen (g/kg)	25.76 *a*	38.83 *b*	42.88 *c*	26.18 *a*
Phosphorus (g/kg)	4.32 *a*	5.80 *b*	7.61 *c*	4.40 *a*
Potassium (g/kg)	41.46 *a*	48.98 *b*	52.42 *c*	41.98 *a*
Sulfur (g/kg)	5.80 *c*	3.58 *a*	3.91 *b*	6.04 *d*
Sodium (g/kg)	2.60 *a*	4.22 *b*	5.01 *c*	2.71 *a*
Calcium (g/kg)	4.84 *c*	2.83 *a*	3.18 *b*	5.52 *d*
Magnesium (g/kg)	3.08 *a*	3.82 *b*	4.14 *c*	3.16 *a*
Chloride (g/kg)	11.13 *a*	16.80 *b*	20.66 *c*	11.28 *a*
Nitrate (g/kg)	1.02 *a*	5.48 *b*	7.63 *c*	0.97 *a*
Copper (mg/kg)	2.80 *a*	4.48 *b*	7.17 *c*	2.78 *a*
Zinc (mg/kg)	20.47 *a*	23.25 *b*	26.13 *c*	20.51 *a*
Manganese (mg/kg)	31.46 *a*	36.28 *b*	41.60 *c*	31.83 *a*
Iron (mg/kg)	156.27 *a*	162.18 *b*	183.06 *c*	155.10 *a*
Boron (mg/kg)	25.25 *a*	25.63 *a*	25.68 *a*	25.38 *a*

The radish were grown in soils amended with autoclaved fish emulsion (treatment 3), inorganic fertilizer (treatment 5), inorganic fertilizer combined with bacteria and actinomycetes (treatment 8) and autoclaved fish emulsion combined with bacteria and actinomycetes (treatment 16). Plants were harvested and analyzed 8 wk after sowing. Values are means of six replicates, and the values with the same letter within a row are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

There were significant ($P < 0.05$) increases in the root and shoot growth characteristics of radish plants grown in soil amended with bacterial and actinomycete isolates and fish emulsion (treatments 9, 10, 12, 13, 15 and 16) compared to those amended with the isolates (treatments 11, 14 and 17) or fish emulsion (treatments 2 and 3) or the inorganic fertilizer (treatments 4 and 5) (Table 1).

The application of the inorganic fertilizer as a soil drench combined with the bacterial mixture (treatment 6) or actinomycete mixture (treatment 7) or the combination of both mixtures of bacteria and actinomycetes (treatment 8) significantly ($P < 0.05$) enhanced the growth and development of radish compared to the treatments which included the application of inorganic fertilizer (treatment 5). However, these treatments performed significantly ($P < 0.05$) less than the treatments which included the application of autoclaved fish emulsion combined with the bacterial mixture (treatment 10) or actinomycete mixture (treatment 13) or the combination of both bacterial and actinomycete mixture (treatment 16) (Table 1).

There were no significant ($P > 0.05$) differences between the root or shoot growth characteristics of radish plants grown in soil treated with either the non-autoclaved fish emulsion (treatment 2) or the autoclaved fish emulsion (treatment 3) (Table 1). In general, there were no significant ($P > 0.05$) differences between the root or shoot growth characteristics of radish plants grown in soil treated with either the non-autoclaved fish emulsion or the autoclaved fish emulsion either singly or in combination with the bacterial and actinomycete isolates. Therefore, only the autoclaved fish emulsion data are presented in Table 1. There were also no significant ($P > 0.05$) differences between the root and shoot growth characteristics of radish plants grown in soil treated with either the inorganic fertilizer applied as a foliar spray (treatment 4) or as a soil drench (treatment 5). In addition, there were no significant ($P > 0.05$) differences between treatments (2 and 3) compared to treatments (4 and 5) without the application of the isolates. Bacterial or actinomycete isolates did not produce any harmful effects on seed germination or subsequent plant growth.

Estimation of the total microbial activity

Microbial activity in soil amended with the autoclaved fish emulsion (treatment 3), was found to be significantly ($P < 0.05$) higher than in soil amended with inorganic fertilizer applied as a soil drench (treatment 5) and non-amended soil (treatment 1) after 4 and 8 weeks from sowing. Over time, there were progress-

Table 3. The effect of soil amendments with autoclaved fish emulsion, inorganic fertilizer applied as a soil drench, autoclaved fish emulsion or inorganic fertilizer in combination with bacteria and actinomycetes on the nutrient levels in the shoots of radish plants grown in an evaporatively cooled glasshouse maintained at 23 ± 3 °C

Nutrients level	Treatment 3	Treatment 5	Treatment 8	Treatment 16
Nitrogen (g/kg)	52.75 *a*	61.01 *b*	65.25 *c*	53.02 *a*
Phosphorus (g/kg)	5.18 *a*	7.21 *b*	8.46 *c*	5.38 *a*
Potassium (g/kg)	46.16 *a*	55.86 *b*	61.36 *c*	46.58 *a*
Sulfur (g/kg)	9.34 *c*	6.21 *a*	7.66 *b*	10.51 *d*
Sodium (g/kg)	3.81 *a*	4.97 *b*	5.75 *c*	3.94 *a*
Calcium (g/kg)	6.25 *c*	4.32 *a*	5.62 *b*	7.63 *d*
Magnesium (g/kg)	3.77 *a*	4.18 *b*	4.84 *c*	3.85 *a*
Chloride (g/kg)	17.34 *a*	20.94 *b*	25.65 *c*	17.47 *a*
Nitrate (g/kg)	4.53 *a*	8.51 *b*	10.32 *c*	4.72 *a*
Copper (mg/kg)	3.48 *a*	6.47 *b*	9.44 *c*	3.56 *a*
Zinc (mg/kg)	24.30 *a*	27.30 *b*	31.03 *c*	24.01 *a*
Manganese (mg/kg)	41.38 *a*	46.63 *b*	53.40 *c*	40.92 *a*
Iron (mg/kg)	183.98 *a*	192.94 *b*	208.96 *c*	184.43 *a*
Boron (mg/kg)	35.12 *a*	35.15 *a*	35.07 *a*	35.23 *a*

The radish were grown in soils amended with autoclaved fish emulsion (treatment 3), inorganic fertilizer (treatment 5), inorganic fertilizer combined with bacteria and actinomycetes (treatment 8) and autoclaved fish emulsion combined with bacteria and actinomycetes (treatment 16). Plants were harvested and analyzed 8 wk after sowing. Values are means of six replicates, and the values with the same letter within a row are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

Table 4. The effect of soil amendments with autoclaved fish emulsion, inorganic fertilizer applied as a soil drench, autoclaved fish emulsion or inorganic fertilizer in combination with bacteria and actinomycetes on the levels of endogenous plant growth regulators (PGRs) in the roots of radish grown in an evaporatively cooled glasshouse maintained at 23 ± 3 °C

*PGRs level (μg 100 g^{-1} dry weight)	Treatment 3	Treatment 5	Treatment 8	Treatment 16
Indole-3-acetic acid (IAA)	20.85 *a*	21.67 *a*	27.62 *b*	32.21 *c*
Indole-3-pyruvic acid (IPYA)	4.69 *a*	4.82 *a*	6.57 *b*	9.83 *c*
Gibberellic acid (GA3)	5.23 *a*	5.33 *a*	8.38 *b*	12.37 *c*
Isopentenyl adenine (IPa)	3.74 *a*	3.86 *a*	5.84 *b*	9.25 *c*
Isolpentenyl adenoside (IPA)	4.53 *a*	4.67 *a*	6.44 *b*	8.38 *c*
Zeatin (Z)	7.24 *a*	7.31 *a*	9.48 *b*	12.11 *c*

The radish were grown in soils amended with autoclaved fish emulsion (treatment 3), inorganic fertilizer (treatment 5), inorganic fertilizer combined with bacteria and actinomycetes (treatment 8) and autoclaved fish emulsion combined with bacteria and actinomycetes (treatment 16). Plants were harvested and analyzed 8 wk after sowing. *PGRs were measured by HPLC, and the values are means of eight replicates. Values with the same letter within a row are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

ive and significant ($P < 0.05$) increases in the total microbial activity of the soil in treatments 1, 3 and 5. Treatment 3 gave an increase of 56% over the 8 weeks period compared to 36% and 8% for treatments 5 and 1, respectively.

Plant nutrient analyses

Plant tissues (both roots and shoots) collected from treatment 8 had significantly ($P < 0.05$) higher nutrient levels than those from plants collected from treatments (3, 5 or 16) (Tables 2 and 3). The concentrations in both roots and shoots of N, P, K, Na, Mg, Cu, Mn, Cl, Zn, Fe and NO_3^-were significantly ($P < 0.05$) higher in treatment 8 than in treatments 3, 5 or 16 (Tables 2 and 3). The concentrations in both roots and shoots of S and Ca were significantly ($P < 0.05$) higher in treatment 16 than in treatments 3, 5 or 8 (Tables 2 and 3). No significant ($P > 0.05$) differ-

Table 5. The effect of soil amendments with autoclaved fish emulsion, inorganic fertilizer applied as a soil drench, autoclaved fish emulsion or inorganic fertilizer in combination with bacteria and actinomycetes on the levels of endogenous plant growth regulators (PGRs) in the shoots of radish grown in an evaporatively cooled glasshouse maintained at 23 ± 3 °C

*PGRs level (μg 100 g^{-1} dry weight)	Treatment 3	Treatment 5	Treatment 8	Treatment 16
Indole-3-acetic acid (IAA)	15.96 *a*	16.37 *a*	20.78 *b*	27. 35 *c*
Indole-3-pyruvic acid (IPYA)	2.71 *a*	2.68 *a*	5.36 *b*	7.41 *c*
Gibberellic acid (GA3)	3.26 *a*	3.38 *a*	5. 84 *b*	9.26 *c*
Isopentenyl adenine (IPa)	2.67 *a*	2.58 *a*	4.40 *b*	7.01 *c*
Isolpentenyl adenoside (IPA)	2.21 *a*	2.25 *a*	4.27 *b*	6.23 *c*
Zeatin (Z)	3.37 *a*	3.30 *a*	5.72 *b*	7.75 *c*

The radish were grown in soils amended with autoclaved fish emulsion (treatment 3), inorganic fertilizer (treatment 5), inorganic fertilizer combined with bacteria and actinomycetes (treatment 8) and autoclaved fish emulsion combined with bacteria and actinomycetes (treatment 16). Plants were harvested and analyzed 8 wk after sowing. *PGRs were measured by HPLC, and the values are means of eight replicates. Values with the same letter within a row are not significantly ($P > 0.05$) different according to Fisher's Protected LSD Test.

ences were evident in B concentrations in root or shoot tissues from these four treatments (Tables 2 and 3).

There were significant ($P < 0.05$) differences in the nutrient levels between treatment 5 compared to treatments 3 and 16 (Tables 2 and 3). On the other hand, there were generally no significant ($P > 0.05$) differences in the nutrient levels between treatments 3 and 16 (Tables 2 and 3). In general, the nutrient levels in the shoots were higher than those of the roots (Tables 2 and 3).

HPLC analysis of PGRs in radish roots, shoots and in culture filtrates of bacterial and actinomycete isolates

Radish grown in soil amended with autoclaved fish emulsion combined with bacterial and actinomycete mixture (treatment 16) had significantly ($P < 0.05$) higher endogenous levels of IAA, IPYA, GA_3, IPa, IPA and Z than treatments 3, 5 or 8 in both roots and shoots (Tables 4 and 5). There were no significant ($P > 0.05$) differences between the PGR concentrations between treatments 3 and 5 (Tables 4 and 5). There were, however, significant ($P < 0.05$) differences between the PGR concentrations between treatment 8 and treatments 3 and 5 (Tables 4 and 5). The levels of PGRs observed in the roots were relatively higher than those of the shoots in treatments 3, 5, 8 and 16 (Tables 4 and 5). In general, the overall PGR levels in both roots and shoots were found to be higher in treatment 16 compared to treatments 3, 5 or 8.

All the isolates tested produced all the PGRs assayed when grown on HFE, whilst some produced only small amounts or failed to produce some of the PGRs when grown on SM. There were significant ($P < 0.05$) increases in the concentrations of IAA, IPYA, GA_3, IPa, IPA and Z produced by bacterial and actinomycete isolates when grown on HFE broth compared to SM. No PGRs were detected in the sterile HFE, SM or modified SM broth.

Discussion

In this study, we established that fish emulsion can be an effective substitute for inorganic fertilizer in the production of radish in the nutrient impoverished sandy soils of the UAE. We also established that the promotion of radish growth by selected bacterial and actinomycete isolates of PGPR can be significantly enhanced by the provision of fish emulsion as their nutrient substrate. In this situation, fish emulsion is not only functioning as a source of inorganic nutrients for plant growth, but also as a substrate for microbially produced PGRs. Fish emulsion has previously been used as a fertilizer for plant growth (Aung and Flick, 1980; Emino, 1981). However, our study is the first to examine the effect of fish emulsion on plant productivity in the presence of beneficial microorganisms.

In the present study, there were no significant differences between the root and shoot growth characteristics of radish grown in soil treated with either the non-autoclaved fish emulsion (NPK 4.5:1.8:1.2) (treatment 2) or the autoclaved fish emulsion (treatment 3) or the inorganic fertilizer (NPK 27:5.5:9) applied either as a foliar spray (treatment 4) or as a soil drench (treatment 5). This is significant considering the relatively larger amount of nutrients available in the inorganic fertilizer. Aung and Flick (1980) reported that soluble nutrients in non-autoclaved fish emulsion applied at weekly or biweekly intervals stimulated vegetative growth and gave comparable growth and fruit yield of greenhouse-grown tomatoes as plants fertilized with full strength Hoagland's nutrient solution. In a similar study, Emino (1981) found that the growth characteristics of seven greenhouse container-grown plant species receiving a weekly application of non-autoclaved fish emulsion (NPK 5:0.44:0.44) was equivalent to the application of a complete inorganic fertilizer solution (NPK 20:8.8:16.6). The results of both studies support our work. Fish emulsion added at 1% by volume was reported to enhance the effect of sawdust waste as a constituent of growth media for tomato resulting in increased yield (Cheng, 1987). In Senegal, Ndiaye et al. (2000) used fish by-products (NPK 5.3:4:0.9) as a soil amendment for growing millet and groundnut and concluded that the application of fish by-products significantly increased millet grain and groundnut productivity compared to the treatments that did not include the fish by-products.

In our study, the fact that resident bacteria naturally present in commercial fish emulsion were not capable of enhancing plant growth on their own, as was evident in the glasshouse trial where there were no significant differences between autoclaved or non-autoclaved fish emulsion treatments may indicate that these resident bacteria are unlikely to be producers of PGRs. However, the large response of plant growth to fish emulsion may indicate that other constituents present in the fish emulsion, in addition to the inorganic nutrients were involved. The chemical composition of fish emulsion and fish meal powder is highly complex and composed of inorganic elements, mixtures of essential amino acids such as histidine, methionine, lysine, serine, tryptophan (Landry and Delhaye, 1994; Miller et al., 1989; Okot, 1995), proteins (Barlow et al., 1981), lipids (Gunstone and Wijesundera, 1978), significant amounts of riboflavin, pantothenic acid, niacin, biotin, folacin and vitamin B-12 (Regier et al., 1974). These constituents are available for uptake by plants through foliage or roots and there is strong evidence indicating the uptake of organic compounds in soil by plant roots (Seear et al., 1968).

In the glasshouse study, the overall PGR levels in plant tissues as well as the yields of plants treated with the combination of bacteria, actinomycetes and the autoclaved fish emulsion (treatment 16) performed significantly better than the other treatments and was followed by plants from treatment 8 and then by plants from treatments 3 and 5. It is noteworthy that treatments 3 (autoclaved fish emulsion) and 5 (inorganic fertilizer) that had similar effects on radish growth also showed similar levels of PGRs in tissues of plants in these treatments. This relationship, however, was not reflected in the levels of overall tissue nutrients. For example, the application of inorganic fertilizer in combination with the mixture of bacteria and actinomycetes (treatment 8) resulted in the highest levels of overall nutrients in tissues, followed by the treatment which included the application of the inorganic fertilizer (treatment 5), whilst the overall nutrient levels of plants grown in soil amended with autoclaved fish emulsion (treatment 3) and autoclaved fish emulsion in combination with the mixture of bacteria and actinomycetes (treatment 16) were similar. This indicates that the significant growth enhancement seen in treatment 16 was more likely to have resulted from the activity of PGRs rather than just from the presence of high levels of plant nutrients. It is likely that the PGRs were able to enhance growth in the presence of adequate mineral nutrients which are also believed to be involved in growth promotion by PGPR (Kloepper et al., 1980).

The treatment which included the application of inorganic fertilizer combined with bacterial and actinomycete mixture (treatment 8) was significantly better than the treatment which included the application of only the inorganic fertilizer (treatment 5) in the glasshouse trial and in the overall PGR production and nutrients analysis. This may indicate that the bacterial and actinomycete isolates may have, in addition to producing growth-promoting substances, helped the plant host in nutrient mineralization and acquisition.

The selected bacterial and actinomycete isolates in the present study produced significant levels of PGRs including auxins, gibberellins and cytokinins in their culture filtrates. Several bacteria and actinomycetes have been reported to produce auxins (El-Abyad et al., 1994; Noel et al., 1996), gibberellins (Gutierrez-Manero et al., 2001) and cytokinins (Al-Desuquy et al., 1998; de Salamone et al., 2001). In our study, the observed plant growth increase with bacterial and actinomycete isolates is supported by other observations where a wide range of free-living bacteria and/or actinomycetes when applied as inoculants are able to enhance germination, plant vigour and plant health (El-Tarabily et al., 1996; Kloepper et al., 1988; Whipps, 2001).

The selection of the PGPR for the glasshouse trial was based not only on their root and rhizosphere colonization potential but also on their ability to produce PGRs critical for radish taproot growth. This certainly helped in the glasshouse evaluation of the fish emulsion in which the bacterial and actinomycete isolates were able to productively utilize PGR precursors. Srinivasan et al. (1996) reported that some *Bacillus* spp. produced significant amounts of IAA when grown in a liquid culture medium supplemented with L-tryptophan, whilst less IAA was produced in a culture medium not supplemented with L-tryptophan. El-Abyad et al. (1994) reported that the addition of DL- tryptophan to a nutrient medium significantly increased production of IPYA by *Streptomyces griseoflavus*. Our work supports these observations, where we found that the concentrations of IAA and IPYA produced by the bacterial and actinomycete isolates tested were significantly higher in the HFE broth compared to SM. This indicates that the HFE broth contains the precursors of IAA and IPYA and probably those of other PGRs.

Fish emulsion has been reported to contain tryptophan, the precursor of IAA (Landry and Delhaye, 1994). The direct uptake by plants of IAA produced by bacteria has also been observed (Libbert and Silhengst, 1970). PGR biosynthesis in soil by rhizosphere micro-organisms may increase upon the addition of their precursors. Nieto and Frankenberger (1989) reported that microbial biosynthesis of cytokinins was enhanced following the application of cytokinin precursors (adenine and isopentyl alcohol) to soil. Sarwar and Frankenberger (1994) also reported that the microbial biosynthesis of auxins was enhanced by L-tryptophan application to soil, resulting in enhanced corn growth.

Our study clearly shows the potential of fish emulsion as a nutrient base for selective microbial inoculation of plants for growth enhancement. Although fish emulsion supported an overall increase in the microbial activity of the soil, its beneficial effect was most pronounced only when appropriate microorganisms were added.

In our glasshouse trial, we used bacteria and actinomycetes and similar tests with fungal isolates are clearly warranted. Very few studies have been made on the relative advantages of various food-bases added with bacterial and actinomycete isolates. Most studies expect all activities of added bacterial isolates to be at the expense of seed and root exudates (Cook and Baker, 1983; Stephens et al., 1993). However, studies with fungi (Connick et al., 1997; Hoitink and Boehm, 1999; Lewis et al., 1996) have considered the value of food-base added with the fungal isolates.

It is noteworthy that the fish emulsion that is commonly used in UAE for the production of horticultural crops at the rates recommended for commercial use, is less expensive than inorganic fertilizers. It is also leached less readily in comparison to inorganic fertilizers and therefore is an attractive substitute or supplement for inorganic fertilizer for horticulture especially in sandy soils. In conclusion, fish emulsion has been shown to serve as a nutrient source for bacterial and actinomycete isolates and for the indigenous micro-organisms promoting the mineralization of complex nutrients into simpler compounds that can be readily taken up by plants as well as by micro-organisms that produce PGRs.

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