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Plant growth promoting effect of 4-quinolinone metabolites from *Pseudomonas cepacia* and 4-quinolinone-3-carboxylate derivatives on red pepper plant (*Capsicum annum)*

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Abstract : Plant growth promoting activity of quinolinone metabolites, 2-(2-hepteny)-3-methyl-4-quinolinone (**1**), 2-heptyl-3-methyl-4-quinolinone, and 2-nonyl-3-methyl-4-quinolinone, produced by *Pseudomonas cepacia* and ethyl 2-methyl-3-alkyl-4-quinolinone carboxylates chemically synthesized were tested by using seed-germination assay, growth increments in plant height after foliar applications. Plant height increment, fresh weight, and the number of fruits were measured after seed-soaking and drench treatment. Compound **1** among the natural products showed a consistent growth promoting effect in seed-germination and plant height after a foliar application. After a seed-soaking and drench treatment, compound **1** and synthetic ethyl 2-methyl-4-quinolinone-3-carboxylate (**5**) showed a significant enhancement in fresh weight and the number of fruits after harvest. Compound **1** and **5** increased the number of fruits per plant by 44% and 84% over the control, respectively (Received March 28, 2002; accepted June 25, 2002).

Key words : seed germination, plant growth promoting effect, foliar application, drench treatment, quinolinone metabolites, *Pseudomonas cepacia*, quinolinone carboxylates, gibberellins.

Introduction

Plant growth regulation is affected by developmental control, responses to stress and nutrient availability, and especially controlled by a variety of different types of compounds including plant hormones and microorganism-oriented secondary metabolites. Plant growth regulatory effect has been known from many natural products; penienone (Kimura *et al.*, 1997), botryslactone (Kimura *et al.*, 1995a), koninginin E inhibiting the growth of etiolated wheat coleoptiles (Parker *et al.*, 1995a; Parker *et al.*, 1995b), pironetin with the growth-inhibitory effect on rice plants without any loss of crop yield (Kobayashi, *et al.*, 1994a; Kobayashi *et al.*, 1994b), spicifernin promoting rice seedling growth (Nakajima, *et al.*, 1990), ampullicin accelerating the root growth of lettuce seedlings by 200% over the control at the concentration of 30 mg/L (Kimura, *et al.*, 1990).

New 4-quinolinone metabolites produced by *Pseudomonas cepacia* strain isolated in the pepper-growing field were reported to show germination-accelerating and growth promoting effect on red pepper plants (Park, 1994; Moon, *et al.*, 1996) and some have been chemically synthesized due to their short natural availability (Moon, *et al.*, 1995). Structural variations on the quinolinone moiety would give different growth

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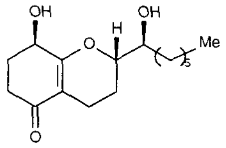
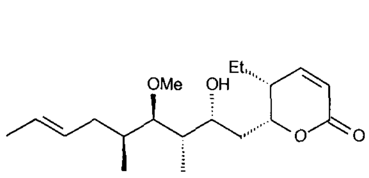
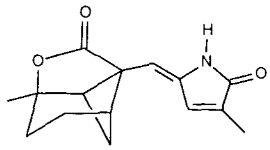


Koninginin E



pironetin



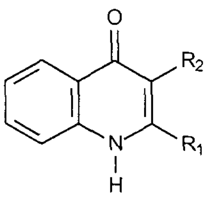
ampullicin

promoting effect. To improve the activity of the natural compounds, an electron-withdrawing group was introduced at the 3-position of 4-quinolinone . Here, the growth promoting effect of 4-quinolinone metabolites (PSC-B, C, and E) from *P. cepacia* and 2-substituted-4-quinolinone-3-carboxylates is reported on red pepper plant (*Capsicum annum*).

Experimental

Chemicals

Quinolinone compounds **1-3** were obtained from the culture media of *Pseudomonas cepacia* (Moon, *et al.*, 1996).



	R_1	R_2
1 (PSC-B)	2-heptenyl	Me
2 (PSC-C)	heptyl	Me
3 (PSC-E)	nonyl	Me
4	Me	CO_2Et
5	Et	CO_2Et
6	Pr	CO_2Et

General Procedure for synthesis of 4-quinolinone-3-carboxylates 4-6

To a solution of isatoic anhydride (5.4 mmole) in dry tetrahydrofuran (50 mL) was added a solution of an anion of ethyl alkyl-3-oxoacetate (6.2 mmole, alkyl = methyl, ethyl, and propyl) which was generated by treatment with lithium diisopropylamide (6.2 mmole, 1.8 M in hexane).

The mixture was stirred at room temperature for 2 h under argon and concentrated under reduced pressure. The resulting yellow solid was washed with hexane, dissolved in a minimum amount of methanol, and left overnight. The resulting crystalline solid was filtered and the yield ranged from 55 to 84%. Physical properties of the products are as follows.

Compound **4** (ethyl 2-methyl-4-quinolinone-3-car boxylate) : mp 175℃ (sublimes); IR (KBr) ν_{max} 2850, 1650, 1595, 1510, 1475, 1445, 1398, 1310, 1255, 1185, 1105, 1000, 795 cm^{-1}; UV (MeOH) λ_{max} 214, 232, 314, 326 nm; ^1H NMR (CD_3OD) δ 8.22 (1 H, *dd*, *J* = 8, 1.6 Hz), 7.70 (1 H, *ddd*, *J* = 8.4, 7.5, 1.6 Hz), 7.53 (1 H, *dd*, *J* = 8.4, 1.0 Hz), 7.40 (1 H, *ddd*, *J* = 8.0, 7.5, 1.0 Hz), 4.37 (2 H, *q*, *J* = 7.2 Hz), 2.52 (3 H, *s*), 1.37 (3 H, *t*, *J* = 7.2) ppm; ^{13}C NMR (CD_3OD) δ 176.9, 168.6, 152.0, 140.6, 133.9, 126.4, 126.0, 125.6, 119.0, 116.3, 62.3, 18.8, 14.6 ppm.

Compound **5** (ethyl 2-ethyl-4-quinolinone-3-car boxylate) : mp 180-185℃ (sublimes); IR (KBr) ν_{max} 2800, 1695, 1565, 1495, 1344, 1290, 1203, 1118 cm^{-1}; UV (MeOH) λ_{max} 214, 234, 315, 327; ^1H NMR (CD_3OD) δ 8.21 (1 H, *br d*, *J* = 8.4 Hz), 7.69 (1 H, *br t*, *J* = 8.4 Hz), 7.56 (1 H, *br d*, *J* = 8.4 Hz), 7.40 (1 H, *br t*, *J* = 8.4 Hz), 4.36 (2 H, *q*, *J* = 7.2 Hz), 2.79 (2 H, *q*, *J* = 7.6 Hz), 1.37 (3 H, *t*, *J* = 7.2), 1.36 (3 H, *t*, *J* = 7.6 Hz) ppm; ^{13}C NMR (CD_3OD) δ 176.1, 167.4, 155.4, 139.8, 132.7, 125.2, 124.7, 124.4, 117.9, 114.7, 61.2, 26.0, 13.3, 13.2 ppm.

Compound **6** (ethyl 2-propyl-4-quinolinone-3-car boxylate) : mp 188-190℃ (sublimes); ^1H NMR (CD_3OD) δ 8.21 (1 H, *dd*, *J* = 8.4, 1.6 Hz), 7.69 (1 H, *ddd*, *J* = 8.4, 8.0, 1.6 Hz), 7.57 (1 H, *dd*, *J* = 8.0, 1.2 Hz), 7.40 (1 H, *ddd*, *J* = 8.4, 8.0, 1.2 Hz), 4.36 (2 H, *q*, *J* = 7.6 Hz), 2.75 (2 H, *t*, *J* = 7.6 Hz), 1.78 (2 H, sextet, J = 7.6 Hz), 1.36 (3 H, *t*, *J* = 7.6), 1.01 (3 H, *t*, *J* = 7.6 Hz) ppm; ^{13}C NMR (CD_3OD) δ 176.1, 167.5, 154.1, 139.7, 132.7, 125.2, 124.8, 124.4, 118.0, 115.1, 61.2, 34.3, 22.9, 13.4, 13.0 ppm.

Seed germination of red pepper

Red pepper seeds (*C. annum*, Dongbuhannong, 3-year old product) were surface-sterilized in 1% sodium hypochlorite solution for 10 min, rinsed with sterile distilled water, and soaked in distilled water for 2 h. The seeds were dried in the shade and placed in the 10-cm Petri dishes (50 seeds/dish) lined with two filter paper discs. Test solutions (6.5 mL, 0.00001, 0.001, 0.1, and 1.0 ppm) were added in the dishes and left in darkness at 25 ℃. After scheduled days (4, 5, 6, and 9 days) the number of germinated seeds were counted (Fig. 1A and B). Test solutions were prepared by dissolving compounds in methanol and diluting it with distilled water with 0.05% of Tween 20. Gibberellin (GA_3, 50, 5, and 0.5 ppm) was used as a positive control standard chemical. Experiments were done in triplicate.

Plant growth after one time foliar application.

Red pepper seeds (Whanggochoo, 3-year old product) were soaked in distilled water and sowed in root media. On 14 days after seeding, plants were transplanted in plastic pots (5 cm in diameter). On 14 days after transplanting, test solutions were applied on the leaves (0.1 m^2) of plants at the concentrations of 10, 1, and 0.1 ppm, which correspond to 20, 2, 0.2 g/ha, respectively. The plant height was measured on 12 and 18 days, after the foliar application. The growth increments were evaluated as a percentage over the height at the beginning of the application (Fig. 2). GA_3 was used as a positive control standard chemical. Experiments were done in ten replicates of thirty pepper plants per each treatment.

Plant growth after two times foliar applications.

Red pepper seeds were sowed in root media. After 14 days, plants were transplanted in a plastic pot (5 cm in diameter). On 26 days after transplanting, the first foliar spray was applied. Test solutions were applied on the leaves of plants at the concentration of 10, 1, and 0.1 ppm, which correspond 20, 2, 0.2 g/ha, respectively. On 6 days after the first foliar application, the second foliar application was done at the same concentrations as the first foliar application. The plant height was measured on 6, 18 and 25 days, after the first application. The growth increments were evaluated as a percentage over the height at the beginning of the first application (Fig. 3). GA_3 was used as a positive control standard chemical. Experiments were done in ten replicates of thirty pepper plants per each treatment.

Fresh weight and the number of fruits after seed soaking and drench treatment.

Seeds were soaked for 5 days at the concentration of 1 ppm except GA_3 (GA_3 was used in 5 and 0.5 ppm). Twelve seeds in a narrow variation in germination effect for 5 days were selected and sowed in a plug tray with 72 cells. Plant height was measured on 20, 33, and 53 days, after seeding (Fig. 4A). Plants were transplanted and drenched with 1 ppm of the test solutions. On 30 and 57 days, after drench, plant height was measured (Fig. 4B). After plants had been grown for 85 days after transplanting, fresh weight per plant and the number of fruits per plant were measured (Fig. 4C and D). Experiments were done in twelve replicates (n = 1).

Results

Red pepper seed germination

The effects of the quinolinone compounds **1-6** on red pepper seed germination were monitored over a nine day germination period (Jun, 1997). Results are presented as a seed germination percentage calculated from germinated seeds divided by a total of seeds tested (Fig. 1). On 6 days, natural product

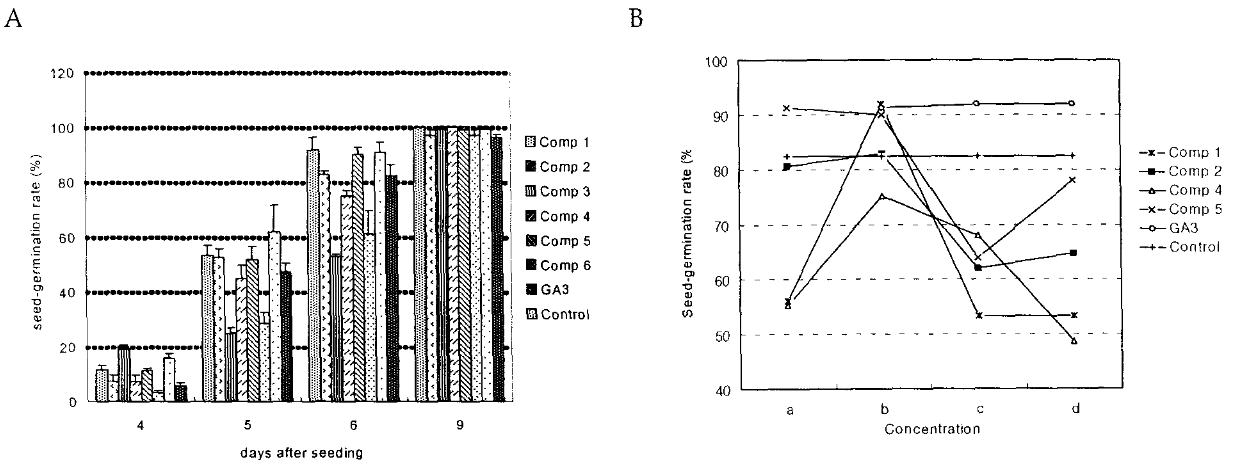


Fig. 1. Effect of 4-quinolinones (1, 2, 3) and 2-alkyl-3-carboxylates (4, 5, 6) on seed-germination of red pepper plants (A) and their concentration dependency (B). (A) The test solutions are at the concentration of 0.001 ppm (GA_3, 0.5 ppm). The results are expressed as a percentage of the germinated seeds over the total number of seeds used. Experiments were done in triplicate. Values are the average of three replicates ±SE (n = 50). (B) The germination rate on day 6 were measured at the various concentrations such as 0.00001 (a), 0.001 (b), 0.1 (c), and 1.0 ppm (d) for the compounds 1, 2, 4, and 5 (for GA_3, b, 0.5; c, 5; and d, 50 ppm).

1 (PSC-B) and compound **5** showed a similar germination effect at the concentration of 0.001 ppm to gibberellin (GA_3, 0.5 ppm) used as a positive control standard chemical (Fig. 1A). However, at higher concentrations they inhibited seed-germination (Fig. 1B). Other compounds (**2**, **3**, and **4**) showed somewhat inhibitory effect, though their activities were at the peak at the concentration of 0.001 ppm.

Plant growth after foliar applications.

Red pepper seeds were sown without soaking with compounds and plants were transplanted. On 14 days after transplanting, test solutions were sprayed on the leaves of plants. On 12 and 18 days, after the foliar application, plant height was measured (Fig. 2).

On 12 days after a foliar application, the growth increments of plants treated with natural product **1** were higher than those of the control, but lower than those of GA_3 at the concentrations of 10 and 0.1 ppm. However, on 18 days, the growth

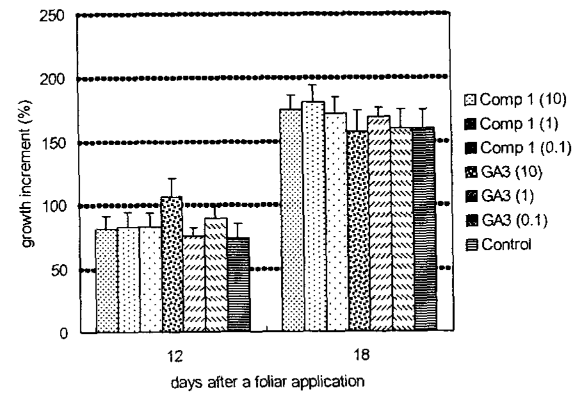


Fig. 2. Effect of PSC-B (1) on the plant growth of red pepper after one time foliar application. On day 14 after transplanting (on day 28 from seeding), a foliar application was done on plants. The applied concentrations are shown in the parentheses in ppm. The results are expressed as a percentage of the growth increment after a foliar spray by the formula $(H_t-H_0)/H_0 \times 100$ where H_t is the height of plants on the days shown and H_0 on the day of the application. Values are the average of ten replicates ± SE (n = 30).

increments for compound **1** were higher than those of GA_3 at the given concentrations. The growth promoting effect of GA_3 was pretty visible in a short period of days after application, but compound **1** gave a long term effect on the growth of pepper plant.

Two times foliar applications on red pepper plant showed growth promoting effect with compound 1 similar to one time application (Fig. 3). After second time application, the growth increments by GA_3, were pretty augmented, while compound 1 did not showed significant increments in growth. The percentages of the growth increments treated with compound **1** were comparable to those of GA_3 at relatively low concentration such as 0.1 ppm, still higher than those of the controls.

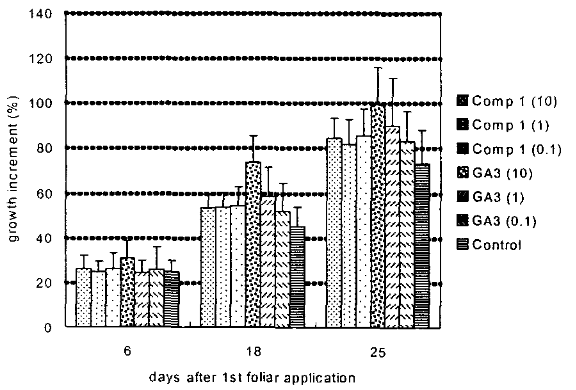


Fig. 3. Effect of PSC-B (1) on the plant growth of red pepper after two times foliar applications. On day 26 after transplanting (after 40 days from seeding), the first foliar application was done on pepper plants. On day 6 after the first application, the second foliar application was done. The applied concentrations are shown in the parentheses in ppm. The results are expressed as a percentage of the growth increment after the foliar sprays by the formula $(H_t-H_0)/H_0 \times 100$ where H_t is the height of plants on the days shown and H_0 on the day of the first application. Values are the average of ten replicates ± SE (n = 30).

Fresh weight and the number of fruits after seed soaking and drench treatment.

The growth-promoting effect of the compounds on seed soaking and drenching treatment was measured on plant height over 53-day period after seeding and 57-day period after transplanting. Fresh weight and the number of fruits per plant were measured over 85-day growing period after transplanting. The concentration of the test solutions was applied at 1 ppm and those of GA at 0.5 and 5 ppm. On day 20, natural products, **1** and **2**, and synthetic compound **4** showed a slightly promoting effect on height, while GA_3 was still giving a best effect (Fig. 4A). Interestingly, on day 20, plants treated with compound **5** was smaller in height than the control, but after 53 days, the effect of compound **5** was negligible in plant height. Natural product **1** significantly promoted plant height all the time, more than GA_3, after seed-soaking and sowing (Fig. 4A). After 53 days, plants were transplanted and drenched with 1 ppm of the test solutions. Plant height was measured after 30 and 57 days after drench treatment (Fig. 4B). Compound **6** showed a considerable effect on the plant height. After plants have been grown for 85 days after transplanting, plants were harvested and the fresh weight and the number of fruits per plant were measured (Fig. 4C and D). Plants treated with compound **5** yielded the highest fresh weight, while compounds **2**, **4**, **6**, and GA_3 (5 ppm) inhibited the growth in weight than the control (Fig. 4C).

Plants treated with natural product **1** yielded 44% more fruits than the control. Surprisingly, plants treated with synthetic compound **5** yielded 84% more fruits than the control, even higher than natural product **1**. Other compounds **2**, **4**, and **6** gave almost the same number of fruits as the control. GA_3-treated plants were not significantly affected in plant fresh weight and the number of fruits, compared to the control.

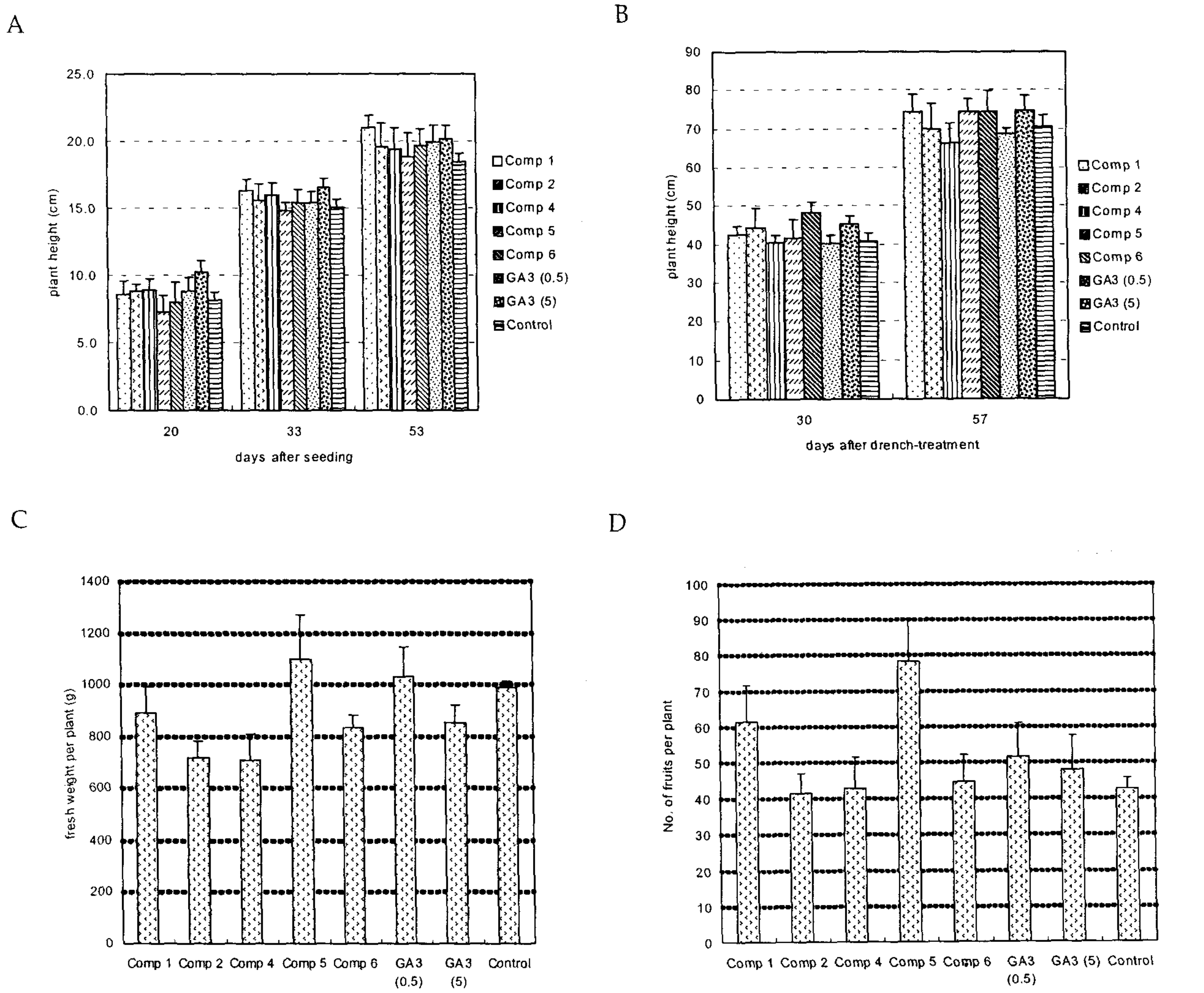


Fig. 4. Effect of quinolinone compounds (1-6) on the plant height of red pepper and on the fresh weight and the number of fruits per plant after the seed soaking and drench treatment. Seeds were soaked for 5 days at the concentration of 1 ppm except GA_3 (GA_3 concentration was shown in parenthesis in ppm) and twelve uniformly-germinated seeds were selected and sowed. Plant height was measured (A) after soaking with the test solutions at the concentration of 1 ppm and (B) after transplanting and drench treatment with 1 ppm of the test solutions. After plants had been grown for 85 days after transplanting, fresh weight (C) and the number of fruits per plant (D) were measured. Values are the average of twelve plants ± SE.

Discussion

The growth-promoting effects of 4-quinolinones metabolites (PSC-B, D, and E) and synthetic 4-quinolinone-3-carboxylates were monitored by using seed-germination assays. All the compounds tested did not show any significant promoting activity on a tobacco seed germination assay in darkness and on a lettuce hypocotyl segment elongation assay (data not shown), but some of them showed a seed-germination effect on red pepper at the concentration of 0.001 ppm, compared to GA_3. Natural product **1** (PSC-B) showed a consistent positive effect over the control on pepper seed-germination (Fig. 1). Also, structurally modified synthetic 3-carboxylate **5** showed a similar effect to compound **1**. However, they inhibited the seed-germination at higher concentrations (e.g., 0.1 and 1 ppm) (Fig. 1B). The quinolinone compounds are believed to be not the kind of hormones like gibberellins or cytokinins. It should be noted that whether or not the compounds actually inhibit germination is uncertain, because methanol which itself somewhat inhibits germination was used in some degree to keep the compounds in solution.

With compound **1** shown the seed-germination effect, a foliar spray was applied on red pepper plants. The growth of plants was significantly enhanced, on day 6 after the foliar application by GA_3. However, after 18 days, the growth increments by compound **1** was equal to or better than GA_3 (Fig. 2). The foliar application effect was reproduced from two times foliar application experiment (Fig. 3). It seemed that GA_3 exhibited an early short term effect on the growth and compound **1** showed a long term persistent effect. Other different effect of compound **1** from GA_3 was that it had an activity to make the leaves grow in darker green and the stem thicker, while GA_3 treatment resulted in leaf-yellowing.

Plants were sown after seed-soaking and grown after drenching with the solution of the compounds to see if the compounds showed a long term effect on fresh weight and the number of fruits after harvest. Natural compound **1** revealed the best effect on the growth increment of plant height after seeding and drench treatment (Fig. 4A and B). It was even better at the concentration of 1 ppm than GA_3 at the concentration of 5 ppm. The fresh weight of plants treated with compound **1** was slightly lower, but the number of fruits was much higher than that of the control (Fig. 4C and D). Synthetic compound **5** with a 3-carboxylic ester group showed seed-germination effect comparable to natural product **1** (Fig. 1) and after seed-soaking it did not show significant growth enhancement in plant height (Fig. 4A). However, after drench treatment with compound **5**, the growth increments in plant height was almost equal to that of compound **1** (Fig. 4B). After harvest, compound **5** showed the most significant influence on the growth promoting effect in fresh weight among the compounds tested including GA_3. The number of fruits per plant reached almost twice over that of the control. The variation in the 3-position of 4-quinolinone compounds to a carboxylic acid ester group from an alkyl group has some influences on the growth promoting activity in the plant height, the fresh weight, and the number of fruits of red pepper plant. It is also notable that structural change at the 2-position of 4-quinolinone also give different effects on the plant growth. The unsaturation and the size in the 2-position of 4-quinolinone moiety as in natural product **1** and compound **5** seem important in the growth promoting effect. The compounds tested showed growth-promoting effect on pepper plant, but not on fruits like melon, grape, and apple. The mechanistic behavior of 4-quinolinone compounds seems unlike that of gibberellins, indole acetic acids, and cytokinins.

Acknowledgements

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Literature cited

Jun, Woo-Bang (1997) International rules for seed testing, International seed testing association. Kor. Turfgrass Sci. 11 (3):211~264.

Kimura, Y., H. Fujioka, T. Hamasaki, K. Furihata, and S. Fujioka (1995) Botryslactone, a new plant growth regulator produced by *Botrytis squamo*. Tetrahedron Lett. 36:7673~7676.

Kimura, Y., H. Nakajima, T. Hamasaki, T. Matsumoto, Y. Matsuda, and A. Tsuneda (1990) Ampullicin and isoampullicin, new metabolites from an Ampulliferina-like fungus sp. No. 27. Agric. Biol. Chem. 54:813~814.

Kimura, Y., T. Mizuno, and A. Shimada (1997) Penienone and penihydrone, new plant growth regulators produced by the fungus, *Penicillium*. Tetrahedron Lett. 38:469~472.

Kobayashi, S., K. Tsuchiya, T. Harada, M. Nishide, T. Kurokawa, T. Nakagawa, N. Shimada, and K. Kobayashi (1994a) Pironetin, a novel plant growth regulator produced by *Streptomyces* sp. NK 10958. I. Taxonomy, production, isolation and preliminary characterization. J. Antibiot. 47:697~702.

Kobayashi, S., K. Tsuchiya, T. Kurokawa, T. Nakagawa, and N. Shimada (1994b) Pironetin, a novel plant growth regulator produced by *Streptomyces* sp. NK10958. II. Structural elucidation. J. Antibiot. 47:703~707.

Moon, S.-S., P. M. Kang, K. S. Yoon, S.-J Yun, and B. B. Park (1995) Synthesis of Plant Growth and Antifungal 4-Quinolinone Metabolites of *Pseudomonas cepacia*, Bull. Korean Chem. Soc. 16:1128~1130.

Moon, S.-S., P. M. Kang, K. S. Park, and C. H. Kim (1996) Plant growth promoting and fungicidal 4-quinolinones from *Pseudomonas cepacia*, Phytochemistry 42:365~368.

Nakajima, H., T. Hamasaki, S. Maeta, Y. Kimura, and Y. Takeuchi (1990) A plant growth regulator produced by the fungus, Cochliobolus spicifer. Phytochemistry 29:1739~1743.

Park, K. S. (1994) Isolation and Identification of Antibiotic Substances from *Pseudomonas cepacia* Antagonistic to *Phytophthora capsici*. Ph. D. Dissertation, Chungbuk National University, Korea.

Parker, S. R., H. G. Cutler, and P. R. Schreiner (1995a) Koninginin E: isolation of a biologically active natural product from *Trichoderma koningii*. Biosci., Biotechnol., Biochem. 59:1747~1749.

Parker, S. R., H. G. Cutler, and P. R. Schreiner (1995b) Koninginin C: a biologically active natural product from *Trichoderma koningii*. Biosci., Biotechnol., Biochem. 59:1126~1127.

_Pseudomonas cepacia_로부터 유래한 4-quinolinone 대사물질과 4-quinolinone-3-carboxylate 유도체의 고추 (_Capsicum annum_)에서의 생장촉진 효과

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요약 : *Pseudomonas cepacia*로부터 유래한 quinolinone 대사물질인 2-(2-hepteny)-3-methyl-4-quinolinone (**1**), 2-heptyl-3-methyl-4-quinolinone, 2-nonyl-3-methyl-4-quinolinone 및 합성물질 ethyl 2-methyl-3-alkyl-4-quinolinone carboxylate의 식물생장촉진 효과를 보기 위해 종자발아검정 및 경엽처리 후의 생장량을 측정하였다. 또한 종자침지 및 관주 처리후의 초고, 생체중, 과실수도 조사하였다. 화합물 **1**은 종자발아 효과와 경엽처리 후에 일관된 생장촉진 효과를 보였다. 종자침지와 관주처리 후에, 화합물 **1**과 합성물질 ethyl 2-methyl-4-quinolinone-3-carboxylate (**5**)를 처리한 식물에서의 생체중과 과실수는 크게 증가하였다. 화합물 **1**과 **5**는 대조군보다 각각 44%와 84%의 과실수 증가를 나타냈다.

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