Role of the auxin, Naphthalene Acetic Acid (NAA) in the pathogenicity of *Sclerotinia sclerotiorum*, causative organism of white mold disease

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Abstract

The effect of Naphthalene Acetic Acid (NAA) on white mold disease was investigated by measuring the mycelium growth rate, and sclerotia weight and number formed by the disease. For this, causative organism *Sclerotinia sclerotiorum* was grown on Potato Dextrose Agar (PDA) amended with different concentrations of NAA. This effect was also investigated on white mold lesion growth rate on bean and cucumber detached leaves, and the devlopment of disease severity on bean and cucumber plants. Results showed that NAA has an inhibition effect on the fungus *in vitro* and it has reduced the weight and the number of sclerotia formation. Consequently, at 200 μg/ml, the mean of mycelium growth rate of three isolate was reduced to 0.4 cm^2/day (=2.9% of mycelium growth rate of control). NAA significantly ($P<0.05$) reduced development of white mold lesions on bean and cucumber detached leaves pretreated concentrations up to 600 μg/ml. Also, it has reduced white mold disease severity on bean and cucumber plants at concentrations up to 500 μg/ml. This effect may be attributed to short-lived partial resistance induced in plant tissues treated with NAA.

Keywords: naphthalene acetic acid, white mold disease, *Sclerotinia sclerotiorum*, and pathogenicity.

الملخص

تم دراسة اثر منظم النمو النباتي النفثالين استيك اسيد (NAA) على تطور مرض العفن الابيض على نباتات الفاصوليا والخيار . حيث تم دراسة اثر المنظم على معدل نمو فطر Sclerotinia sclerotiroum المسبب لمرض العفن الابيض على ثلاث مستويات : البيئة المغذية، الأنسجة النباتية والنباتات الكاملة وأظهرت النتائج ما يلي:

أدى استخدام الأوكسين (NAA) بتركيزات لغاية 500µg/ml على تقليل معدل نمو الفطر، معدل تكوين الاجسام الحجرية الخاصة بالفطر وعددها فى الوسط المغذي والأنسجة النباتية، وأظهر الاوكسين مقدرة في تقليل نسبة الاصابة بالمرض بنسب 54%، 27% على محصولي الفاصوليا والخيار بالترتيب عند استخدامه بتركيز 400 µg/ml وعزي ذلك الى مقدرة الأوكسين على إحداث مناعة ذاتية مؤقتة في الأنسجة النباتية.

Introduction

The role of plant growth regulators in pathogenesies of plant disease is not widely studied. But, there is an increasing evidence that both of the pathogen and host plant have the capacity to synthesize various growth regulators, and alterations in their levels and/or sensitivity as a result of plant pathogen interaction. These levels are related to disease susceptibility or resistance reaction (Singh *et al*., 1997). Research work on these subjects has provided new insights into our understanding of pathogenesis and manipulation of disease resistance (Singh *et al*., 1997). Some investigations indicated that NAA is a potential antifungal agent (Nakamura *et al.*, 1978; Tomita *et al.*, 1984; Michniewicz & Rozej, 1988). The role of auxins in growth and development of fungi is not yet clearly elucidated. Working with *Neurospora crassa*, Japanese researchers have demonstrated that auxins may act as regulators of conidial germination (Nakamura *et al.*, 1978) and growth of mycelium (Tomita *et al.*, 1984). According to these workers, auxin may control fungal cell elongation and sporulation and that auxin may act as regulator of growth and differentiation in fungi-like organisms and higher plants. Further studies on the role of auxin in the growth and development of *Fusarium culmorum* were conducted *in vitro*. The results indicated that auxin slightly stimulated mycelial growth and spores production at low concentrations, and at 10^{-5} M concentration, it strongly inhibited mycelial growth and sporulation, in addition to spore germination. (Michniewicz & Rozej, 1987).

Naphthalene Acetic Acid (NAA), Indole Acetic Acid (IAA), 2,4-dichlorophenoxy acetic acid (2,4,D) and Abscisic acid (ABA) were exogenously applied in North America to control early blight of potato (*Alternaria solani*), (Melinda & Stevenson 1991). The results indicated that NAA, 2,4-D produced accurate quantitative resistance to early blight when applied at 10.7 mM and 4.0 mM, respectively.

Sclerotinia sclerotiorum is an important plant-pathogenic fungus causing white mold disease, a serious yield-limiting disease of many crops in many countries (Steadman, 1983). *S. sclerotiorum* is the causal agent of white mold disease in bean and cucumber plantations in winter and spring in many Palestinian agricultural fields. The control of white mold disease is not an easy task especially when cool, moist conditions occur during flowering. The present work was aimed at investigating the role of Naphthalene Acetic Acid in the interaction between *S. sclerotiorum* as a plant pathogen and their host plants, namely, bean (*Phaseolus vulgaris*) and cucumber (*Cucumis sativus*), in an attempt to explore their potential in controling white mold disease.

Materials and Methods

The role of NAA (Fine Agrochemical, England) in host-parasite interaction was evaluated in the system of *Sclerotinia sclerotiorum* against bean (*Phaseolus vulgaris*) and cucumber (*Cucumis sativus*) *in vitro*, *in vivo*, and on entire host plants.

Isolates

Isolates of *S. sclerotiorum* used in this study were either recovered from infected bean and cucumber plants, or collected from various greenhouses and open fields located in different Palestinian agricultural areas.

At least 25 pure isolates cultured on potato dextrose agar (PDA) amended with 250 mg/L chloramphenicol, and kept in skimmed milk in deep freez at -30ºC. Only three isolates of *S. sclerotiorum* (SS.P10, SS.18, and SS.P 26) had been used in all experiments.

Effect of Naphthalene Acetic Acid *in vitro*

PDA medium (Oxoid CM-139) was prepared as follows: 39 g/L and 250 mg/L chloramphenicol (Sigma C-0378) were suspended in deionized water and dissolved by stirring on a magnetic hot plate. 250 ml of medium was transferred into 300-ml flasks and autoclaved at 121ºC, 15 psi for 15 minutes.

Growth regulators were incorporated in PDA medium after autoclaving (set on shaking water bath at 42 ºC) to give final concentrations of the active ingredient (μg/ml) of NAA: 0, 1, 10, 50, 100, 150, and 200.

From each concentration 250 ml auxin-amended culture medium were dispensed into eighteen, 90-mm diameter petri plates (~14ml per dish) and allowed to solidify. Five mm diameter mycelial plugs of six-day old cultures of *S. sclerotiorum* isolates (SS.P10, SS.I18, and SS.P26) were used to inoculate 6 replicate plates for each isolate. The plates were then incubated under dark conditions at 22ºC. Colony diameter was measured at 44, 68, and 74 hours and sclerotia number & weight were measured after 3 weeks. Mycelium growth rate (cm^2/day) in the plates was calculated using the following equation:

$$\mathbf{R = \{[(D/2)^2 - (d/2)^2)] X p\}/T -}$$

where R, mycelium growth rate; D, average diameter of colony (cm); d, disc dimeter (cm); p = 3.14, and T, time of incubation (day).

Bean and cucumber detached leaves

Two week-old bean seedlings (cv. Hilda) and three week-old cucumber seedlings (cv. Dalellia) were replanted in 15*30 cm (width *height) pots placed in a glasshouse. The planting medium is comprised of a mixture of peatmoss and perlite (2:1, V/V). Plants were irrigated and a 20:20:20

NPK fertilizer was added twice a week. At flowering stage (40 days after planting date). Healthy young leaves were collected and placed with the upper side down on the bottom of plastic boxes (40x25x15cm), over a plastic mesh platform. The plastic mesh was placed a on sterilized wet towel paper to preserve enough humidity in the box. Each box had either six bean or cucumber leaves.

The experimental design used was completely a randomized design (CRD), where each six bean leaflets or three cucmber leaves were considered as replicates for each treatment in each box. The leaves were sprayed with Naphthalene Acetic Acid solution using a microsprayer. The concentrations (μg/ml) of NAA active ingredient solution used were as follows: 0, 200, 300, 400, 500, and 600.

After having absorbed the NAA solution, the detached leaves were inoculated by placing a 5-mm diameter agar block, taken from a 6-day-old culture of three isolates of *S. sclerotiorum* (SSP.10, SSP.26, and SSI.18) growing on PDA, on the lower surface of the leaflet. Six replicates were used for each isolate per box. The boxes were moistened, covered by transparent plastic film and incubated at 22ºC with 12 hours photoperiod.

Evaluation of disease development on bean detached leaves was carried out by careful examination of rotting leaf area around the inoculum disk at 93 hours by measuring the lesion diameter.

Disease growth rate was calculated using the equation mentioned above.

Bean and cucumber plants

Two-week-old bean seedlings and three week-old cucumber seedlings were replanted in 15*30 cm pots in a mixture of peatmoss and perlite (2:1 v/v). Plants were irrigated and fertilized with the commercial fertilizer (20:20:20 plus trace elements) twice a week. Twelve, 40-day old bean plants and 30-day old cucumber plants for each concentration, were sprayed until saturation by 30ml/plant of NAA: 0, 200, 400, and 600 μg/ml active ingredient. After two hours, time needed for absorption of NAA solution absorption, evrey each 4 plants, which was previously treated with every concentration of NAA was inoculated with homogenized mycelium suspended in a deionized sterile water containing (g/L) 2 glucose, and 1 potassium dihydrogen phosphate (KH_2PO_4) of one of three isolates. Each plant was sprayed with 20 ml of mycelium suspension of *S. sclerotiorum* isolates (SS.P10, SS.I 18, and SS.P26). Plants were covered with transparent plastic sacks to maintain humidity, and they were incubated in a growth chamber at 22ºC, with 12 hours of illumination.

The development of white mold disease was evaluated by estimation of the disease severity (%) at 11 days. Disease severity was rated on the

basis of a 0-100% scale, where 0= no visible symptoms; 1-10% = small, circular and irregular grewish spots on lower leaves; 11-20% = leaf symptoms enlarged as lesions on lower leaves; 21-30% = small, circular and irregular grewish spots began to appear on upper leaves; 31-40% = leaf symptoms enlarged as lesions on upper leaves; 41-50% = rot symptoms appeared on leaves, petiols and stem ; 51-60% = wilt brownish lesions clearly appeared on leaves , petiols and stem; 61-70%= white mold mass began to appear on 50-60% of the entire plant; 71-80% = wilted leaves defoliated; and 81-100% = plants completely wilted and died.

Experimental design was completely randomized, It considered every each 4 plants inoculated with mycelium suspension of one of three isolates as replicates for each treatment.

Statistical analysis

The results of the effect of NAA on mycelium growth rate, sclerotia weight and number; white mold lesions growth on bean and cucumber detached leaves, and on white mold disease severity (%) a on bean and cucmber whole plants were analyzed statistically using one-way analysis of variance (ANOVA) to test for significance, and the Tukey test was used for means separation by Sigma Stat. Software Program (1997)

Results

Effect of NAA on S. sclerotiorum in vitro

Auxin, napthalene acetic acid (NAA), significantly (P<0.05) decreased the mycelium growth rate, sclerotia weight and number of three isolates of *S. sclerotiorum* (SS.P10, SS.I18, and SS.P26) growing on PDA at 1, 10, 50, 100, 150, and 200 μg/ml concentrations compared to the control. At 200 μg/ml, the mean of mycelium growth rate of three isolate was reduced to 0.3 cm^2/day (=2.9% of mycelium growth rate of control). This inhibitory effect increased significantly with the increasing of NAA concentration (Fig.1, Table1).

The effect of NAA on white mold disease development *in vivo*

The development of white mold disease on cucumber and bean detached leaves pre-treated with different concentrations of NAA was evaluated by measuring disease lesions growth rate (cm^2/day) 4 days after inoculation. Therefore, NAA significantly (P<0.05) reduced development of white mold lesions on both types of detached leaves pretreated with 200, 300, 400, 500, and 600 μg/ml of NAA (Fig 2). No significant differences in lesions area developed on detached leaves of bean caused by three isolates at concentrations of 200, 300 and 400 μg/ml, were detected. Increase of the concentration did not also affect significantly lesions area.

At concentrations of 500 and 600 μg/ml, significant variations were observed between the isolates. In addition, a white mold lesion induced by isolate SSP.26 was significantly larger than that induced by isolates SSP.10 and SSI.18. Moreover, chlorosis was observed on bean leaves treated with 600 μg/ml of NAA (Fig 2,Table1).

The effect of NAA on white mold severity on bean and cucumber plants

Development of white mold disease on entire plants of bean and cucumber previously treated with different concentrations of NAA was estimated visually as whole plant disease severity (%) after 11 days of incubation (Fig. 3).

NAA significantly ($P<0.05$) reduced mean white mold disease severity (%) at 200, and 400 μg/ml of bean plants. Differences between the virulence of different isolates at the same concentrations were not significant. At 600 μg/ml NAA, white mold severity on bean for three isolates was not affected. The next higher concentration also caused leaf chlorosis. Mean disease severity in bean plants was significantly higher at 600 μg/ml compared to that at 200 and 400 μg/ml. Cucumber white mold disease severity was significantly ($P>0.05$) reduced at 400 and 600 μg/ml compared to the control (Fig 3, Table 1).

Discussion

Results obtained revealed that NAA reduced mycelium growth rate, sclerotia weight and number of *S. sclerotiorum in vivo* at concentrations < 200 μg/ml due to the suppressive effect of the auxin on the fungus. It also reduced fungus lesion development *in vivo* and white mold disease severity on bean and cucumber entire plants at concentration up to 600 μg/ml. Similar results were observed by Michniewicz & Rosej (1987) who reported that NAA at 10^{-5}M strongly inhibited mycelium growth, sporulation, and spore germination of *Fusarium culmorum in vitro*, whereas it increased spore production and germination at low concentrations (10^{-7} and 10^{-9} M). NAA, exogenously applied at 10.7 mM, and 4.0 mM, was found by Melinda & Stevenson (1991) to increase the resistance of potato plants to early blight incited by *Alternaria solani*. The present results are also in partial agreement with those of Gruen (1959) who indicated that the auxin was not a growth and development regulation factor in fungi, but it sometimes stimulated fungal growth under unfavorable conditions. Many reports e.g., Michniewicz & Rosej (1987) and Melinda & Stevenson (1991) have pointed out that auxin acts as a growth and development controlling factor in fungi, and this role may vary in different species. The velocity and flux of auxin transport vary according to many factors, including nature or type of auxin, and maturity of tissues. NAA circulates slowly in the plant tissue; it is slower

than other auxins, and the flux transport rate of NAA in bean tissue is slow (Elnora & Frank, 1978). So, when NAA is exogenously applied on bean and cucumber plant tissues, it accumulates in the upper cell layer where it initiates a short- lived partial resistance against the fungus. Also, the fungus *S. sclerotiorum* penetration through tissue is enhanced by enzymes capable of degrading the middle lamella of the host cell. Optimum pH-value range of enzymes pectinase, pectinmethylesterase (Lumsden, 1976), cellulases, hemicellulases (Baraki-Golan, 1974; Lumsden, 1976), phosphatidase (Lumsden, 1970), and proteolytic enzymes (Khare and Bompeix, 1976) are 4.3 - 5.5. The pH-value of NAA spray solutions used in this study was 6.63 - 7.69 depending on concentrations. This may reduce the enzyme activity and thus the penetration of *S. sclerotiorum* into host cells is also reduced. NAA increased white mold disease severity on bean plants at 600 μg/ml compared to lower concentrations when exogenously applied, propably because it stimulates endogenuos ethylene production in plant tissue at high concentrations (Saniewski et al., 1990). In turn, the exogenous application of NAA at 600 μg /ml, and then endogenous production of ethylene, propably enhanced the protein synthesis (Abeles, 1969), caused phytotoxic, chlorosis of bean plant tissues, and increased the penetration of fungal mycelium.

The results realved that naphthalene acetic acid (NAA) reduced white mold disease severity on bean and cucumber plants at certain concentration 200-400 μg/ml due to inhibition effect on fungus and induction of short-lived partial resistance in NAA treated plant tissues. Further research on the effect of NAA on white mold disease under field conditions, however, is still needed.

References:

1. Abeles, F.B., (1969). Auxin stimulation of ethylene evolution. *Plant Physiology,* 41: 585-588.
2. Baraki-Golan, R., (1974). Production of cellulase and polygalacturanse by *Sclerotinia minor*. *Mycopathology* 54, 297-301.
3. Elnora A. Schneider and Frank Wightman, (1978). Auxins. In: Phytohormones and Related Compounds,ed. By Letham, G., and Higgins. Elsevier, Notrth Holland biomedical press.
4. Khare, K. B, and Bompeix, G. (1976). Activities proteolytiques des *Sclerotinia sclerotiorum* et *Sclerotinia minor*: role possible de la pathogenese. *Annual Reviw Mycology* (Abstract) 40, 65-66.
5. Lumsden, R. D., (1970). Phosphatidase of *Sclerotinia sclerotiorum* produced in culture and in infected bean. *Phytopathology* 60, 1106-1110.
6. Lumsden, R. D., (1976). Pectolytic enzymes of *Sclerotinia sclerotiorum* and their localization in infected bean. *Canadian Journal of Botany* 54, 2630-2641.
7. Melinda, J., Stevenson, W.R., (1991). A leaf disk assay for detecting resistance to early blight caused by *Alternaria solani* in juvenile potato plants. *Plant Disease*. 75: 385-389.
8. Michnievicz, M., and Rozej, B., (1988). Is the gibberellin limiting factor for the growth and development of *Fusarium culmorum*? *Acta Physiologiae Plantarum*. 10: 227-236.
9. Michniewicz, M., and Rozej, B., (1987). Further studies on the role of auxin on the growth and development of *Fusarium culmorum* (W.G.Sm.) Sacc. *Acta Physiologiae Plantarum*, 9: 219-227.
10. Nakamura, T., Kawanabe, Y., Takiyama, E., Takahashi, N. and Murayama T., (1978). Effects of auxin and gibberellin on conidial germination in *Neurospora crassa*. *Plant Cell Physiology*, 19: 705-709.
11. Saniewski, M., Kawa, L and Wegrzynowiz, E., (1990). Influence of different concentrations of auxin and thiosulfate on stem growth and ethylene production in tulips. *Biology Science,* 38:51-56.
12. Sigma stat. software program (1997).
13. Singh, P.P., A.S. Barsa, and P.P.S. Pannu (1997). Abscisic acid is a potent inhibitor of growth and sporidial formation in *Neovosia indica* cultures: dual mode of action via loss of polyamines and cellular turgidity. *Phytoparasitica* 25: 111-116.
14. Steadman, R., (1983). White mold- a serious yield- limiting disease of bean. *Plant Disease*, 67: 346-350.
15. Tomita, K., Murayama T. and Nakamura, T., (1984). Effects of auxin and gibberellin on elongation of young hyphae in *Neurospora crassa*. *Plant Cell Physiology*, 25: 355-358.

Table 1 Effect of NAA on: A- mycelium growth rate (cm^2/day) of three isolates of *S. sclerotiorum* grown onPDA-amended with different concentration of NAA; B- on white mold lesions growth rate on bean leaves; C-on white mold lesions growth rate on cucumber leaves; D- on bean white mold disease severity (%) and E- on cucumber white mold disease severity. All incubated at 22°C.

Conc. (μg /ml)	Isolate SS.P10	Isolate SS.I18	Isolate SS.P26
		A (PDA+NAA)	
0	13.62*****±1.5 a******	13.54±1.2 a	12.39±1.1a
1	8.36 ± 0.9 b	7.85± 0.7 b	10.16± 0.6 b
10	5.93 ±1.4 c	6.6± 1.0cg	7.65± 0.5cg
50	2.58± 0.9d	3.12± 0.2d	3.96±1.3 c
100	0.75± 0.2ef	1.00± 0.18 ef	1.06± 0.16 ef
150	0.59± 0.2ef	0.37± 0.11 ef	0.37± 0.2ef
200	0.40±0.08 ef	0.33± 0.12 ef	0.08 ± 0.17ef
		B (bean leaves)	
0	6.2±1.0 a	5.9±1.6 a	5.7±3.0 a
200	1.6± 0.6 bd	1.3± 0.3 bd	1.6± 0.3 bd
300	1.1± 0.5 bd	1.1± 0.2 bd	1.0± 0.4 bd
400	1.0± 0.3 bd	0.8± 0.2 bde	1.0 ± 0.5 bd
500	0.1± 0.1 ce	0.5± 0.2 ce	1.0± 0.3 cd
600	0.1± 0.1 ce	0.4± 0.1ce	0.7± 0.2 ce
		C (Cucumber leaves)	
0	2.9± 0.5 a	3.3± 0.3 a	3.7± 0.3 a
200	1.8± 0.2 bc	1.6± 0.4 bc	1.6± 0.2 bc
300	1.3± 0.4 bc	1.1± 0.2 bc	1.1± 0.5 bc
400	0.9± 0.2 bcd	0.8± 0.2 bcd	0.7± 0.07 bcd
500	0.3± 0.2 bcd	0.3± 0.1 bcd	0.4± 0.2 bcd
		D (% disease severity on bean)	
0	72.5 ±5.6 a	60 ±3.5 a	60 ±7.9 a
200	36.7 ±2.3 bc	46.2 ±6.2 b	43.7 ±4.7 b
400	37.5 ±2.9 bc	33.7 ±9.4 bc	32.5 ±6.9 bc
600	63.8 ±4.8 a	60 ±4 a	62.5 ±2.9 a
		E (% disease severity on bean)	
0	37 ±8.3 a	33.7 ±9.6 a	30 ±6.1 a
200	30 ±11.5 a	27.5 ±8.6 a	30 ±9.1 a
400	27.5 ±8.7 a	22.5 ±2.8 b	22.5 ±2.8 b
600	23.7 ±4.8 b	21.2 ±2.5 b	18.7 ±2.5 b

****** Means followed by the same letter within a column or row for each NAA treatment are not statistically significant according to Tukey multiple comparison test (P>0.05). *Mean of six replicates ± standard deviation.

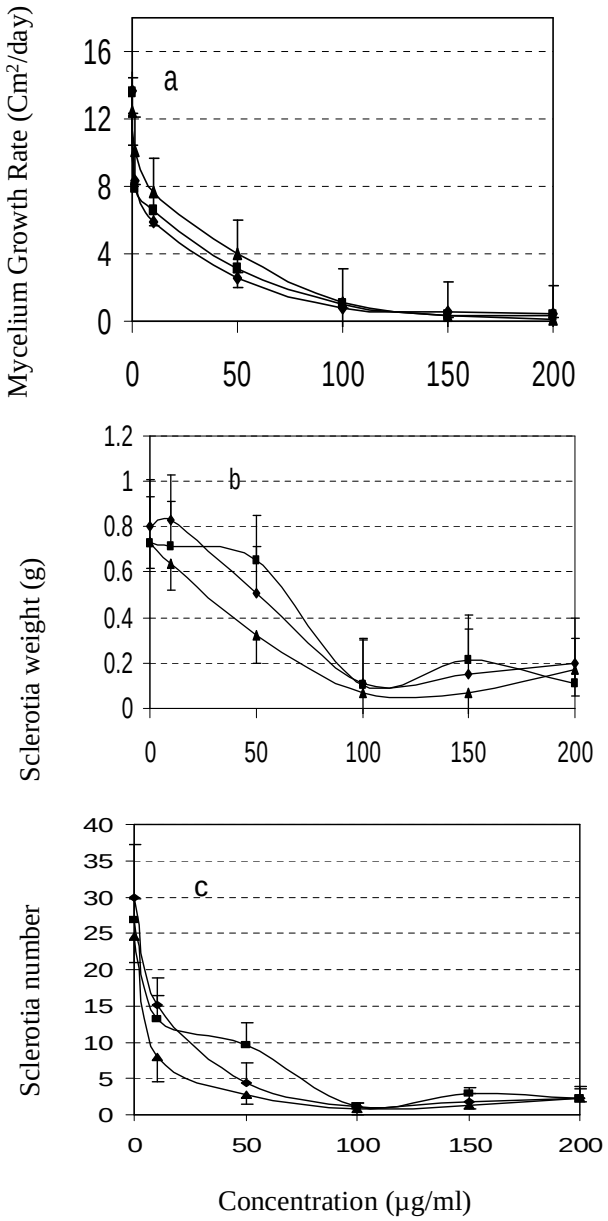

Figure 1 Effect of naphthalene acetic acid (NAA) on mycelium growth rate (cm^2/day)(a), sclerotia weight(b), and sclerotia number of three isolates SS.P.10 (-◆-), SS.I.18 (- -), SS.P.26 (- -) of S. sclerotiorum grown on PDA-amended with different concentration of NAA incubated at 22°C.

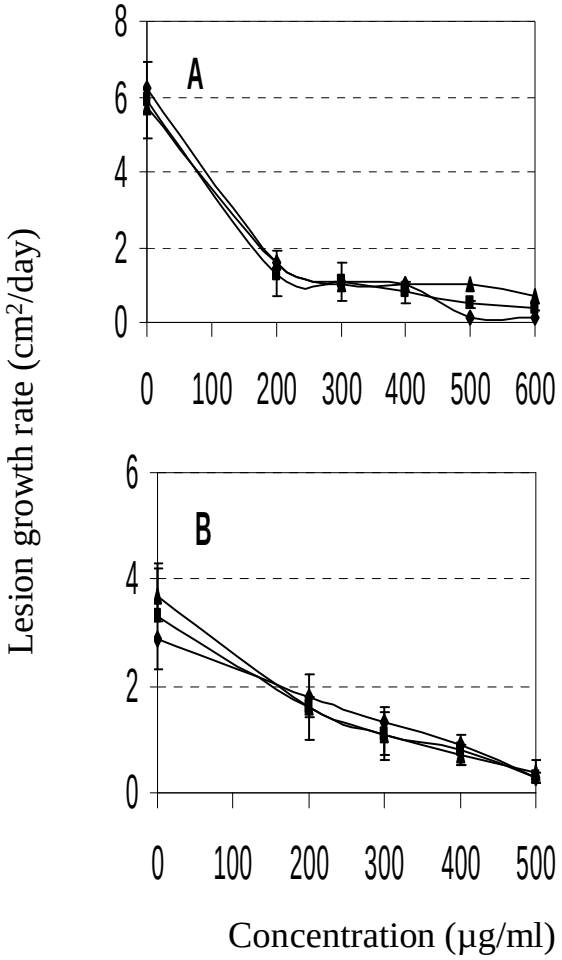

Figure 2 Effect of naphthalene acetic acid (NAA) on white mold lesion growth rate (cm^2/day) caused by three isolates SS.P.10 (-◆-), SS.I.18 (- -), SS.P.26 (- -) of *S. sclerotiorum* on bean (A) and cucumber (B) detached leaves after 4-days incubation period at 22ºC.

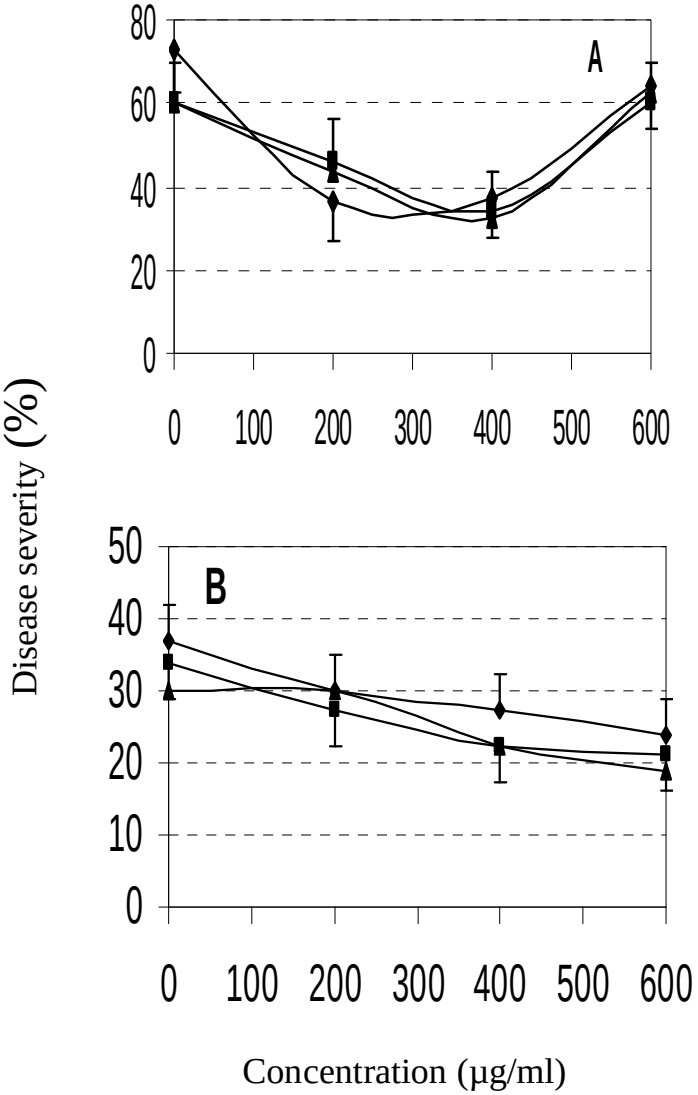

Figure 3 Effect of naphthalene acetic acid (NAA) on white mold disease severity (%) caused by three isolates SS.P.10 (-◆-), SS.I.18 (- -), SS.P.26 (- -) of *S. sclerotiorum* on bean (A) and cucumber (B) plants pretreated with different concentration of NAA after 11 days incubation at 22°C.