Annual REPORT

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Submitted to the Office of the Science Advisor
U S Agency for International Development

Project Title
Oligosaccharides as a natural Fungicides against
Diseases caused by Phytophthora and Alternaria
in Potato and Botrytis in Tomato

Project Number TA- MOU-95-CA15-005

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Project Number TA -MOU-95-CA15-005

A I D Grant project Officer Dr John A Daly

Project duration From January 1, 1996, to November 30, 1999

Executive Summary

The purpose of the project for 1998 was to evaluate the potential of the chitin oligomers to elicit the defense system of the plants The leaves of the cucumber and bean plants were sprayed with the oligomers Four different enzymes chitinase , glucanase ,chitosinase and peroxidae which suppose to be part of the defense response of the plant were analyzed The enzymes were studied colorimetric and by gel electrophoresis techniques Results has been shown that chitin oligomers induce differently the different enzymes and the same enzyme can elicit differently in different tissues (draft of the paper with all the results are included) Chitosan a glucoseamine polymer was also studied in this project in order to compared its elicitation response and latter on its effect on the disease control The effect of the chitin oligomers and the glucoseamine polymer on the disease control will be discuss in the final report In this year we trained Dr Natalia Kudabeava in our lab Her work is still continue and the results will appear in the final report Dr Natalia is the only worker which dealing with this project in her country In this case no other work has been done in her country We feel that only in the end of the project Dr Natalia is ready for independent work in her country

B

Section I

Research objectives and Research accomplishments

are part of the paper that we are included

Scientific Impact of Collaboration

Dr Natalya kudabeava will finish to spend in our lab at February more than one year of training We feel and she feel that it was a very fruitful time for her During this year Dr Rouza Kunaeva the Kazakh chief of the project visited in our lab

Description of the Project impact

We will discuss this issue in the final report after the invivo experiments

Strengthening of Developing country institution

The project enable the molecular biology peoples to train themselves with field experiments The project allow them to combine experiments in the lab with experiments in the greenhouse

Further work

We are doing this year experiments with the whole plants in order to test the effect of the chitin oligomers in the greenhouse In the final report we will discuss this issue

C

Section II

Managererial issue Not arise any msnsgerial issues

Budjet No major changes

Special concerns not applicable

Collaboration ,Travel ,Training and publications

Dr kuneava the head of the kazakh group visited in our lab She spend one week in our lab for discussion the progress of the project and for thinking about a new proposal We are facing a very successful training with Dr Natalia Kudabaeva She is still working in our lab and will continue to work at her lab with the treated raw material that she prepared The full details will be in the final report , at the end of this year

We include the draft of the paper which will give much more details on the aim of our work

Elicitation Effect of Chitin Oligomers and Chitosan Spraying on the Leaves of Cucumber (*Cucumis sativus*) and Bean (*Phaseolus vulgaris*) Plants

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ABSTRACT

The objectives of this research were to evaluate the elicitation of enzymes involved in the plant defense mechanism such as chitinase, peroxidase, β-1,3 glucanase and chitosanase, caused by spraying chitin oligomers (oligomer) and chitosan on cucumber (*Cucumis sativus*) and bean (*Phaseolus vulgaris*) plants The elicitation response was evaluate by colorimetric and gel electrophoresis techniques Two chitinase isoforms were observed in a native gel in cucumber leaves while five to six chitinase isoforms were observed in bean leaves The basal peroxidase activity was enhanced up to 142 3% in cucumber leaves 48 h after the treatment with an oligomer mixture However, in chitosan treated plants an increase When the plants were treated by chitosan, an increase of 113 3% was detected in bean plants while the increasing was only 22 6% in cucumber plants The basal glucanase activity was increased (107 6%) in bean leaves of chitin oligomer treated plants, while only 16 5% in cucumber leaves In cucumber and bean plants treated with the chitosan the glucanase elicitation was not very effective Under denaturing gel conditions, a band with of 45 kDa apparent molecular weight was detected for glucanase in both plants The chitosanase activity was elicited 135% in cucumber and 161 7% in bean leaves 48 h after oligomer treatment of the plants, while after the chitosan treatment the elicitation in both plants was similar (94 - 96%) Also under denaturing gel conditions chitosanase isoenzymes with the same size (ca 30 kDa) were observed for both plants

These results suggest that chitin oligomers and chitosan elicit different responses in the cucumber and bean plants

INTRODUCTION

Chitinases [EC 3 2 1 14] and β-1,3 glucanases [EC 3 2 1 39] are present in many higher plants Both enzymes have been implicated in defense reactions of plants against pathogens **(Boller et al , 1983)** β-1,3 glucanases and chitinases are differentially induced by exogenously supplied mercuric chloride in soybean hypocotyls and leaves **(Yi and Hwang, 1996)** Further, oligosaccharides that are released from the fungal cell walls by the actions of chitinases and β-1,3 glucanases are thought to act as signals (elicitors) for further of induction of these enzymes and for activation of other defense mechanisms such as phytoalexin production and lignification **(Mauch and Staehelin, 1989)** Chitinase and β-1,3 glucanase isozymes can be quantified by using PAGE, and protein bands can be stained with Coomassie Brilliant Blue on the same gels after staining for the isozymes **(Pan et al , 1991)**

Peroxidases [EC 1 11 1 7] are part of the multi-component defense system **(Malolepsa and Urbanek, 1994)**, peroxidases are involved in catalyzing the chitosanization of cinnamyl groups to lignin **(Lagrimni et al , 1993)**, suberization of cell walls **(Sherf et al , 1993)**, accumulation of phenolic chitosans **(Graham and Graham, 1992)** and the binding of extensin to the cell wall **(Brownleader et al , 1995)** Systemic accumulation of peroxidase can be induced by treatment of leaves with K_2HPO_4 **(Irving and Ku , 1990) Hammerschmidt et al , (1982)** found that increase in peroxidase activity was associated with an enhancement of three acidic peroxidase isoforms

Various molecular forms of chitosanase activity were found in leaf tissue of various plant species stressed with chemicals or pathogens by using polyacrylamide gel assays **(Grenier and Asselin, 1990)** Chitosanases [EC 3 2 1 99] are hydrolases acting on chitosan They are distinguished from chitinases by their lower apparent molecular mass [after sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), under non-reducing conditions] and substrate specificity **(Grenier and Asselin, 1990)** Chitosanase activity in leaves, seeds and fruits were investigated in several species Chitosanase activity was found also in the leaf extracts of lower plants such as *Onoclea sensibilis* (fern) as well as in several higher plants **(Quakfaqui and Asselin, 1992)**

Chitosan, a high molecular weight cationic polysaccharide, has been shown to be fungicide against several fungi **(Hirano and Nagao, 1989)** Chitosan (poly (1→4)-β-D-glucosamine) can be defined as deacetylated chitin **(Grenier and Asselin, 1990)** Chitosan also is known to be a potential elicitor of many plant defense responses, including the accumulation of chitinases **(Mauch et al , 1984)**, lignification in wheat leaves **(Pearce and Ride, 1982)**, and induction of callose synthesis **(Kauss et al , 1989)** Different kind of plants tested for elicitation response under the chitosan treatment some of these treatment were applied on detached leaves **(Tiuterev et al , 1996)**, or spraying on the various plant species leaves as on leaves of *Phaseolus Vulgaris* cv Pinto **(Pospieszny et al , 1991)** Injection of chitosan into leaves of *Nicotiana tabacum* Xanthi nc induced systemic resistance against TMV **(Pospieszny, H , 1995)** In tomato plants the chitosan was applied by leaf spraying or root coating for increase the resistance **(Benhamou and Thériault, 1992)**

The present study investigates whether chitin oligomers and chitosan spraying on the leaves of cucumber and bean plants affect the elicitation the plant defense mechanism

MATERIALS AND METHODS

Chemicals

Chemicals for electrophoresis were from Bio-Rad (Mississauga, Ontario, Canada) Protein molecular mass markers, glycol chitosan, laminarin, 2,3,5-triphenyl tetrazolium chloride, 3-amino-9-ethylcarbazol, coomassie brilliant blue R-250, calcofluor white MR2, triton X-100 and all other reagents were from Sigma Chemical Co (St Louis, MO, USA) Glycol chitin was obtained by acetylation of glycol chitosan by the method of **Molano et al , 1979** Chitin oligomers were prepared as described Chitosan was purchased from

Plant Material

Cucumber (*Cucumis sativus)* cv Hasan plants were grown in plastic pots containing potting mixture in a commercial nursery ('Hishtil', Afula, Israel) Bean (*Phaseolus vulgaris*) plants were grown from seeds under greenhouse conditions For the elicitation response, cucumber and bean plants were treated in the 3-4 true leaf stage Control plants were sprayed with water and treated plants were sprayed with 0 1% chitin oligomers mixture (DA 95%, DP 2-7) or 0 1% chitosan (DA 7%, MW 300,000)

Preparation of leaf extract

Leaves of cucumber and bean plants were harvested and immediately deeping in liquid nitrogen, freeze dried and then milling in a mortar and pestle to a fine powder For leaf crude extract, the leaves powder (0 1 g) was extracted in 5 ml sodium phosphate buffer (0 05 M, pH 6 5) for 2 h at 4°C, then centrifuged at 20,000 g for 15 min at 4°C After centrifugation, the supernatants were collected and their protein concentrations were determined by the method of Bradford using bovine serum albumin (BSA) as a standard [5]

Native polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gel electrophoresis under native conditions was performed with 15-12% or 8% (w/v) polyacrylamide resolving and 5% (w/v) stacking gels Anodic electrophoresis was carried out according to the method of Davis, 1964 [8] Gels were stained with 0 01% calcofluor white for detection of chitinase and chitosanase activities, with 0 15% 2,3,5-triphenyl tetrazolium chloride for glucanase activity, or with 1% 3-amino-9-ethylcarbazol for peroxidase activity Cucumber and bean leaf crude extracts (25-30 μg protein) were loaded in the activity gels All the gels were run using the Mini protean II apparatus (Bio-Rad) at 100 volts for 2 h

Detection of chitinase activity after PAGE

Chitinase activity was detected in 12% polyacrylamide resolving and 5% stacking gels Glycol chitin (0 01%) was used as the substrate in the gel matrix After electrophoresis, the gels were incubated at 40°C for 3 h in 0 1 M sodium acetate buffer (pH 5 0) with gentle shaking **(Trudel and Asselin, 1990)** Following incubation chitinase activity gels were stained at room temperature with freshly prepared 0 01% (w/v) calcofluor white M2R in 0 1 M Tris-HCl (pH 8 9) buffer for 8 min Calcofluor white solution was removed by washing the gels in distilled water for 24 h The chitinase activity bands were observed in the UV light screen as a dark bands on the fluorescent background

Detection of Peroxidase activity after PAGE

Peroxidase activity was detected in 8% resolving and 5% stacking gel matrix After PAGE, gels were rinsed with distilled water and transferred to 0 05 M sodium acetate buffer (pH 5 0) containing 10 mM H_2O_2 for 2 min Then, 3 ml of N,N-dimethylformamide containing 30 mg 3-amino-9-ethylcarbazol was added with slow shaking Brown bands appeared in 10 min **(Shimoni, 1994)** Gels were rinsed

with distilled water several times and once with 0 05 M sodium acetate buffer (pH 5 0) solution Then, the gels were placed into the fixation solution (25% methanol, 10% acetic acid, 5% glycerol) or stained with Coomassie R-250

Denaturating - PAGE

SDS-PAGE was performed with 15% (w/v) polyacrylamide resolving and 5% stacking gels containing 0 1% SDS (w/v) Cucumber and bean leaf crude extracts (25-30 μg protein) were loaded in the activity gels All the gels were run using the Mini protean II apparatus (Bio-Rad) at 100 volt for 2 h Anodic electrophoresis was carried out according to the method of Davis, 1964 [8] Samples were boiled for 3 min with SDS loading buffer **(Promega Protocols)**

Detection of β-1,3 glucanase activity after denaturing PAGE

β-1,3 glucanase activity was detected in SDS-PAGE Laminarin (0 4%) was used as the substrate in the gel matrix After SDS-PAGE, the gels were incubated at room temperature for 10 min in 0 05 M sodium acetate buffer (pH 5 0) containing 1% (v/v) purified Triton X-100 and then the Triton X-100 solution was removed The gels were rinsed three times with distilled water, stained with 0 15% 2,3,5-triphenyl tetrazolium chloride in 1 M NaOH solution in a boiling water bath, until red bands appeared **(Pan et al , 1989)** After staining, the gels were soaked in a solution of 7 5% acetic acid and stored at 4°C

Detection of chitosanase activity after denaturing PAGE

Chitosanase activity was detected in SDS-PAGE Glycol chitosan (0 02%) was used as substrate in the gel matrix After electrophoresis, the chitosanase activity gels were first incubated at room temperature for 20 min in 0 01 M sodium acetate buffer (pH 5 0) containing 1% (v/v) purified Triton X-100 **(Grenier and Asselin, 1990)** and then the Triton X-100 solution was removed and the gels were incubated at 40°C for 4 h in 0 01 M sodium acetate buffer (pH 5 0) with gentle shaking Detection of the enzyme activity was done by staining with calcofluor white as described for detecting of chitinase activity Chitosanase activity bands were observed in the UV light screen as a dark bands on the fluorescent background

Colorimetric assay for measurement of chitinase, peroxidase, β-1,3 glucanase and chitosanase activities

Chitinase activity was measured using 0 4 ml assay mixture containing 0 2% glycol chitin and enzyme solution containing 40 μg protein Glycol chitin is useful for the colorimetric assays because it does not precipitate at any pH **(Aiba, S , 1992)** The mixture was incubated at 40°C for 1 h Then, 0 5 ml of ferricyanide (0 5 g potassium ferricyanide in 1 liter of 0 5 M sodium carbonate) was added and the mixture was immediately boiled for 15 min After cooling, the chitinase activity was determined as the release of the N-acetyl-glucosamine (Glc-NAc) group at 420 nm according to **Imoto and Yagishita (1971)**

Peroxidase activity was assayed spectrophotometrically The 1 ml assay mixture containing 0 05 M sodium acetate buffer (pH 6 0), 0 2 ml of 0 1 M pyrogallol, 0 1 ml of 90 mM H_2O_2 and enzyme solution containing between 10-40 μg protein, mixed together immediately before detecting The activity was measured at 300 nm according to **Kanner and Kinsella, 1983** The reaction was allowed to proceed for 6 min with OD measurements taken every 60 s, beginning after 1 min after the adding of the crude extract to the substrate **(Lurie et al , 1997)**

β-1,3-glucanase activity was colorimetrically assayed by the dinitrosalicylate method of Abeles and Forrence **(Abeles and Forrence, 1979)** with some modifications The 0 2 ml assay mixture was incubated at 40° C overnight Crude leaf extracts containing 40 μg protein were assayed using 2% dialyzed laminarin as a substrate

Chitosanase activity was assayed as described for the β-1,3-glucanase using 0 2% glycol chitosan as the substrate and enzyme solution containing 40 μg protein The 0 2 ml assay mixture was incubated at 40° C overnight The reaction was stopped by adding 0 8 ml of 0 03 M dinitrosalicylate reagent and the 1 ml final reaction mixture was immediately boiled for 10 min **(Pan et al , 1989)** After cooling, the resulting reducing sugars from glucanase and chitosanase activities were measured at 575 nm using the method of Nelson, 1944 []

RESULTS

Detection of enzyme activity by polyacrylamide gel electrophoresis (PAGE)

The activities of chitinase, peroxidase, glucanase and chitosanase enzymes were checked under the native and denaturing gel conditions, in the leaf crude extracts of cucumber (*Cucumis sativus)* and bean (*Phaseolus vulgaris*) plants 48 h after the treatment with chitin oligomers and chitosan was found most active time for the four enzyme activities (data were not shown)

Induction of Chitinase

Chitinase isoforms were detected in native gel containing 0 01% glycol chitin as substrate Under this conditions, oligomer treated plants showed stronger chitinase activity bands in both plants (***Fig 1 A, columns 2,5***), than the control (columns 1,4) and chitosan treated plants (columns 3,6) In cucumber were found only two basic chitinase isoforms, while in bean were found more than six chitinase isoforms (Fig 1A)

Induction of Peroxidase

Peroxidase isoforms were detected under native PAGE conditions In cucumber plant, chitin oligomer treated samples (Fig 1B, column 2) showed stronger peroxidase activity than the water (column 1) and chitosan treated plants (column 3) Under this conditions, three different peroxidase isoforms were detected in cucumber plants

In bean plant, not very significant differences were observed between the treatments It seems that in chitin oligomer treated plants was found higher activity The cucumber (columns 1-3) and bean (columns 4-6) peroxidase isoforms were located at different sizes on the activity gel (***Fig 1 B***)

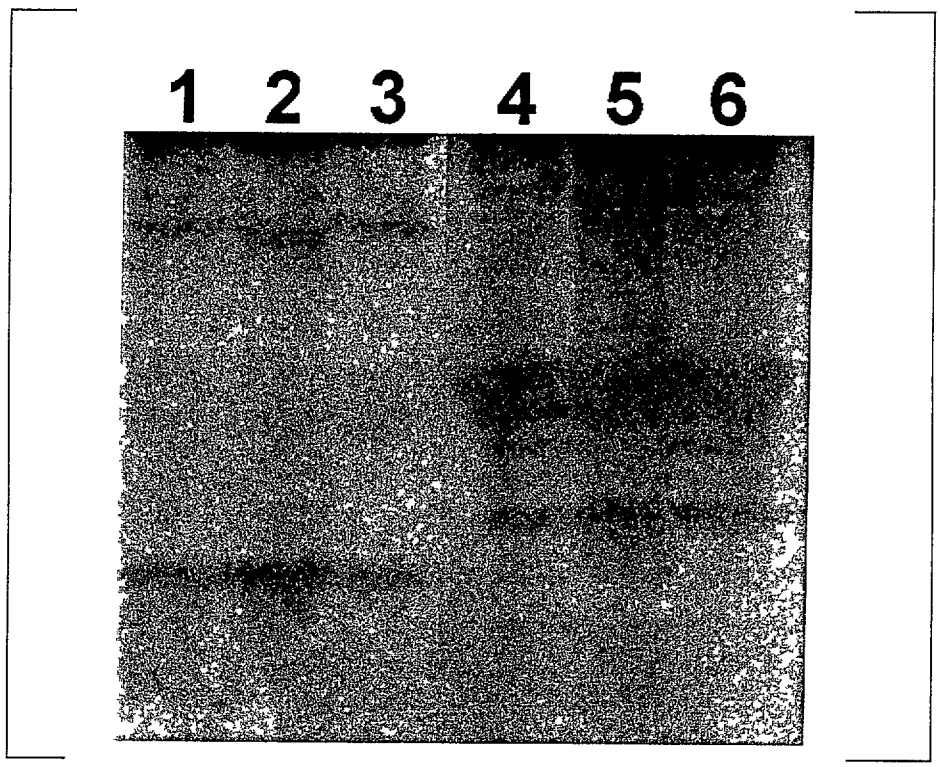
Induction of β-1,3-glucanase

In bean and cucumber plants, chitin oligomer treated plants (columns 2,5) showed higher β-1,3-glucanase activity than the control (1,4) and chitosan treated plants (3,6) as shown in ***Fig 1 C*** However, the induction of β-1,3-glucanase activity in bean plants was stronger than in cucumber plants Only one major band of β-1,3-glucanase activity near 45 kDa was observed for both plants

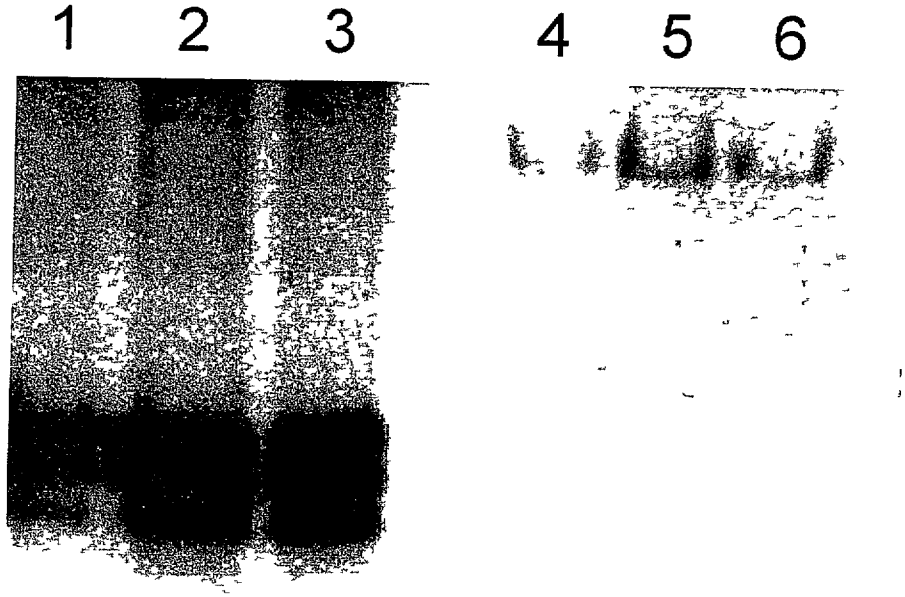
Induction of Chitosanase

When the cucumber plants were treated by chitin oligomers (column 2) and by chitosan (column 3), a weak induction in chitosanase activity bands were observed In bean plants a stronger induction in chitosanase activity bands were observed (columns 5,6) In this conditions both plants showed chitosanase activity bands around 30 kDa (***Fig 1 D***)

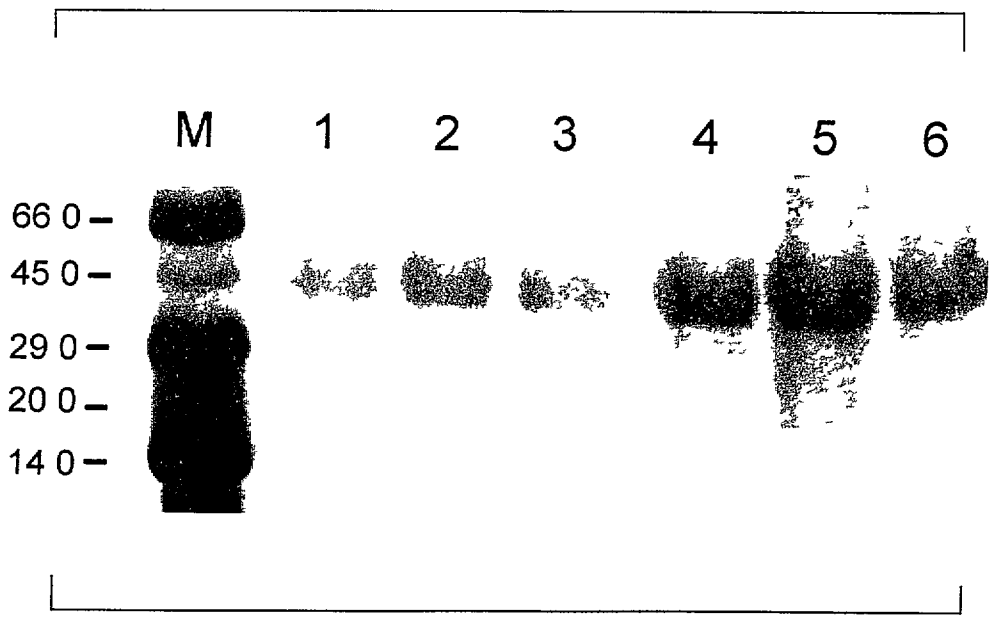
A



B



C



D

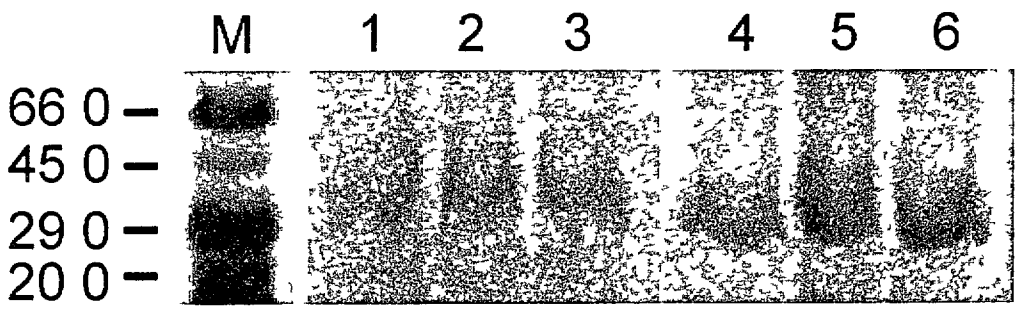


Fig 1 **A Chitinase** activity after 12% (w/v) native polyacrylamide gel electrophoresis gels were stained with calcofluor white M2R ***B*** **Peroxidase** activity after 8% (w/v) native polyacrylamide gel electrophoresis gels were stained with 3-amino-9-ethylcarbazol ***C*** **β-1,3-Glucanase** activity after 15% (w/v) denaturing (SDS) polyacrylamide gel electrophroresis gels were stained with 2 3 5-triphenyltetrazolium chloride ***D*** **Chitosanase** activity after 15% (w/v) denaturing (SDS) polyacrylamide gel electrophroresis gels were stained with calcofluor white M2R Leaf crude extracts (25-30 μg of protein) were loaded per lane For determing the molecular sizes for β-1 3-glucanase and Chitosanase in the denaturation gels 10 μl of molecular mass markers denaturated with SDS (**M**) ***Cucumis sativus,*** (lane 1) control (lane 2) treated with chitin oligomer (lane 3) treated with chitosan (chitosan) and ***Phaseolus vulgaris,*** (lane 4) control, (lane 5) treated with chitin oligomer (lane 6) treated with chitosan

Detection of enzyme activity by colorimetric methods

The activities of chitinase, peroxidase, glucanase, and chitosanase that are involved in the plant defense mechanism were tested in cucumber (*Cucumis sativus)* and bean (*Phaseolus vulgaris*) plants 48 hours after the treatment with chitin oligomers and chitosan In both plants the activities of these four enzymes were found to be most active 48 hours after the treatment (data not shown)

Induction of chitinase activity

The basal chitinase activity founded in cucumber leaves was 2 81 mg NAG/mg Prot/ml While in bean leaves higher basal activity was found (4 57 mg NAG/mg Prot/ml)

The chitinase enzyme activity was weakly induced in cucumber plants treated with the chitin oligomers mixture to 3 31 (17% increase) was observed (Fig 2) However, in cucumber plants treated by the chitosan the activity was 1 9 (32% decrease) While in bean leaves both treatments caused a decrease in the activity (48% in chitosan treated plants and 16 % in oligomer treated plants) (fig 2)

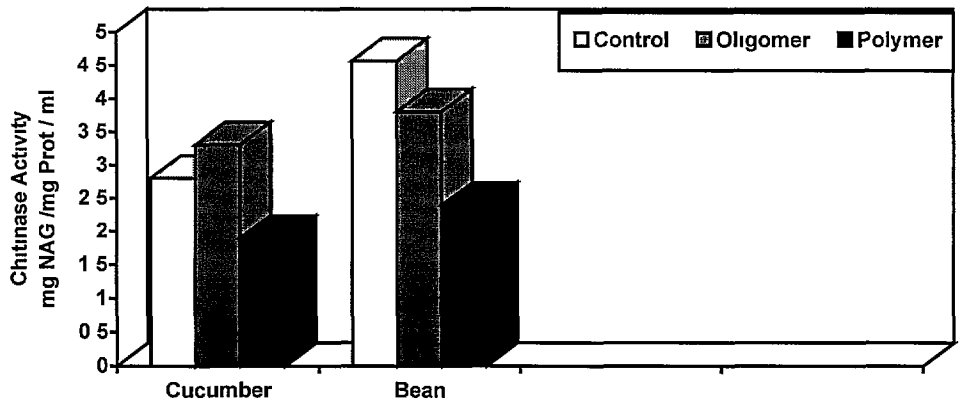


Fig 2 Chitinase activities according to the colorimetric results in cucumber (*Cucumis sativus)* and bean (*Phaseolus vulgaris)* leaves The white column showes water treated gray column showes 0 1% chitin oligomer mixture treated and black column showes 0 1% chitosan treated plants

Induction of peroxidase activity

The basal peroxidase activity founded in cucumber leaves was 59 63 OD_{300nm}/mg Prot/ml

Furthermore, after the chitin oligomer treatment the activity increased to 144 5 (142 3% induction) in cucumber leaves However, when the plants were treated with chitosan, the activity raised to 73 1 (22 6% induction) only as shown in Fig 3

In bean leaves basal chitinase activity was 23 64 OD_{300nm}/mg Prot/ml After both treatments the activity increased to ca 50 43 OD_{300nm}/mg Prot/ml corresponding to almost 110% induction (Fig 3)

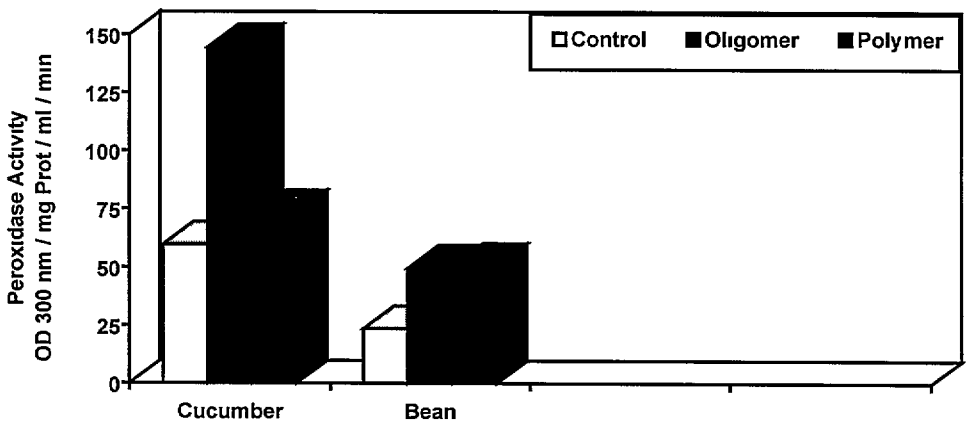


Fig 3 Peroxidase activities according to the colorimetric results in cucumber and bean leaves The white column showes water treated gray column showes 0 1% chitin oligomer treated and black column showes 0 1% chitosan treated plants

Induction of β-1,3 glucanase activity

The basal glucanase activity in cucumber plants was 7 92 μg glucose/mg Prot/ml The glucanase activity in cucumber leaves was slightly elicitated in chitin oligomer and in chitosan treated plants, 16 5 and 32 3% increase, respectively as shown in Fig 4

In bean leaves, basal glucanase activity founded was 13 59 μg glucose/mg Prot/ml The glucanase activity was enhanced to 28 22 (107 6% induction) but only to 19 59 (44% increase) by chitosan (Fig 4)

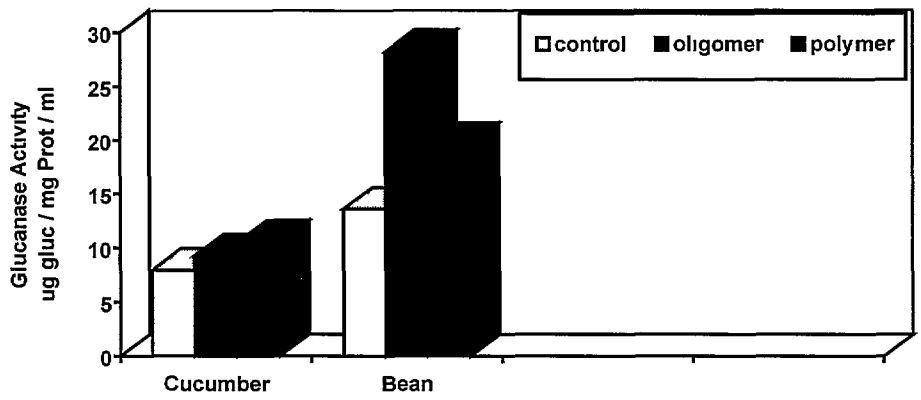


Fig 4 β-1 3 glucanase activities according to the colorimetric results in *Cucumis sativus* and *Phaseolus vulgaris* leaves The white column showes water treated gray column showes 0 1% chitin oligomer treated and black column showes 0 1% chitosan treated plants

Induction of chitosanase activity

The basal chitosanase activity founded in cucumber leaves was 1 97 µg glucose/mg Prot/ml While in bean leaves basal chitosanase activity was much lower, 0 47 µg glucose/mg Prot/ml

The activity of chitosanase was significantly elicitated in both plants leaves by either the chitin oligomer and chitosan treatments In cucumber leaves, the enzyme activity raised to 4 63 by chitin oligomer (135% induction) and by the chitosan to a value of 3 83 (94 4% increase) (Fig 5) Similar elicitation response by chitin oligomer (161 7%) and by chitosan (95 7%) were found in bean leaves as shown in Fig 5

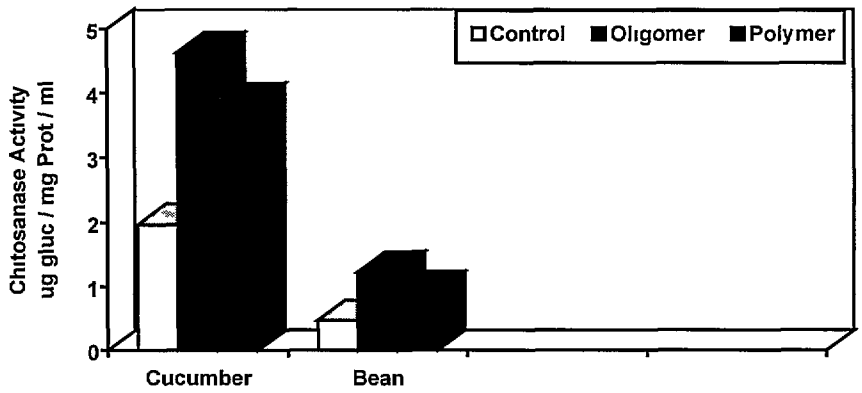


Fig 5 Chitosanase activities according to the colorimetric results in *Cucumis sativus* and *Phaseolus vulgaris* leaves The white column showes water treated gray column showes 0 1% chitin oligomer treated and black column showes 0 1% chitosan treated plants

DISCUSSION

We have reported that chitinas, peroxidase, glucanase, and chitosanase can be elicitate by chitin oligomers and by chitosan in cucumber and bean plants These four enzymes are known to participate in early defense mechanism against pathogens It has been shown that chitosan amendment of the nutrient solution stimulated chitinase, chitosanase and β-1,3 glucanase activity in roots and leaves of cucumber plants **(Ghaouth et al , 1994)** In strawberry fruit, coating intact strawberries with chitosan did not stimulate chitinase, chitosanase or β-1,3-glucanase activities in the tissue as revealed by PAGE assays Chitosan, when applied on freshly cut strawberries, however, stimulated acidic chitinase activity **(Ghaouth et al , 1992)** The action af chitosan on the resistance of potato plants were tested by **Tiuterev et al , (1996)**, the upper leaves of plants were detached and placed into moist chambers and were sprayed by chitosan solution Also it has been shown that β-1,3 glucan oligomers can induce chitinase activity in rice suspension cultures **(Inui et al , 1997)** Elicitation of plant defense mechanisms by chitosan also has been demonstrated in other systems **(Kendra et al , 1989)** Induction of chitinase and β-1,3 glucanase activities and the elicitation of phytoalexins by chitosan also have been reported in excised pea pods

(Nichols et al , 1980, Kendra et al , 1989) According to **Leuba and Stossel, (1986)** the activity of chitosan is related to its ability to interfere with the plasma membrane function

According to our gel electrophoresis results, in cucumber leaf crude extracts two different chitinase isoforms were observed in activity gels while bean leaf crude extracts were showing more than six chitinase isoforms Cucumber and bean samples showed stronger chitinase activity bands when the plants were treated by chitin oligomers mixture, while control and chitosan treated plants' leaves showed the same activity in the activity gel Also in soybean, pathogen infection and chemical stress has been found as a function in a different organ-specific manner to induce isoforms of β-1,3-glucanase and chitinase **(Yi Yon and Hwang, 1996)** According to our colorimetric results, the response of chitinase elicitation showed a weak increasing in cucumber plants by oligomer treatment In bean plants, no elicitation was observed In both plants, the chitosan treated plants showed higher activity than in oligomer treated plants by colorimetrically These results did not supported by the PAGE results because gel systems are more sensitive than the colorimetric measurements

Peroxidase activity measurements by gel electrophoresis, in cucumber leaves three acidic peroxidase isoforms were observed while in the bean plants only one peroxidase isoform was observed under this conditions The bean leaves peroxidase was observed at different location than the cucumber enzyme Similar three acidic peroxidase isoforms in the cucumber leaves were already published **(Hammerschmidt et al , 1982)** Also a similar set of acidic peroxidases that were also systemically induced in watermelon and muskmelon has been reported **(Smith and Hammerschmidt, 1988)** The size isoforms from these three cucurbits species has been located in a range of 30 to 33 kDa Leaf extracts from oligomer treated cucumber and bean plants had stronger activity bands in activity gel In cucumber plants treated with chitosan higher activity of the three isoenzymes was also observed Colorimetrically, peroxidase activity was very strongly elicitated in cucumber plants by chitin oligomer but when the plants were treated by chitosan the elicitation response was weaker However, in bean plants both treatments caused a 2 fold increasement in peroxidase activity There was a corelation between gel and colorimetric results in the bean plants but not in the cucumber plants

Glucanase activity measured by gel electrophoresis conditions only one major band was observed close to 45 kDa in cucumber and bean plants Also **Ji and Kuc , (1995)** has reported only one isoform detected in cucumber leaf extracts and molecular mass was apparent near 38 kDa close to our results The glucanase basal activity in bean leaves was higher than in cucumber leaves Only in oligomer treated bean plants higher glucanase activity was founded In cucumber leaves increase in the glucanase activity by chitin oligomers or chitosan was observed **Ghaouth et al , (1994)** showed that, there was no β-1,3-glucanase activity detected in the leaf intercellular fluid extracts from control cucumber plants in native gels It has been shown that among the glucanase isoforms present in the roots, only the lower band appeared to increase in intensity with a higher chitosan concentration When we looked to the colorimetric results, glucanase activity was slightly enhanced in cucumber plants by both treatment But in bean plants, the activity was increased more than 2 fold after the chitin oligomers treatment when elicitation response was not very strong by chitosan The same results were shown by denaturing PAGE also

Chitosanase activity bands were observed around 30 kDa in cucumber and bean leaf extracts In cucumber plants chitosanase activity bands was not very clear **Ghaouth et al , 1994,** had been reported about four chitosanase activity bands in leaf intercellular fluid extracts of cucumber plants which grown in presence of chitosan at 100 or 400 μg/ml for 14 and 17 days Colorimetrically, the elicitation of chitosanase activity was stronger after chitin oligomer treatment in both plants, than by chitosan

In the light of our results and these observations, elicitation response were found in changeable values by gel electrophoresis and by colorimetric methods in cucumber and bean leaves when we sprayed the plants with chitin oligomers and chitosan According to other researchers, they were mentioned wounding, detaching or adding these oligomers or chitosan to the extract solutions But it was not clear, if the treatment is the reason for the elicitation response whether from wounding stress But in our study, whole cucumber and bean plants were sprayed with chitin oligomers and chitosan and the elicitation response was observed by different techniques Correlation between the PAGE and colorimetric results was not allways observed for every enzyme activities, but the increasing in the elicitation response exist

We conclude that either oligomers and chitosans can be use as natural products, because of their nontoxic effects on the environment, for elicitation of plant defense mechanism

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