Expression of Chitinase Gene in *Escherichia coli*

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by

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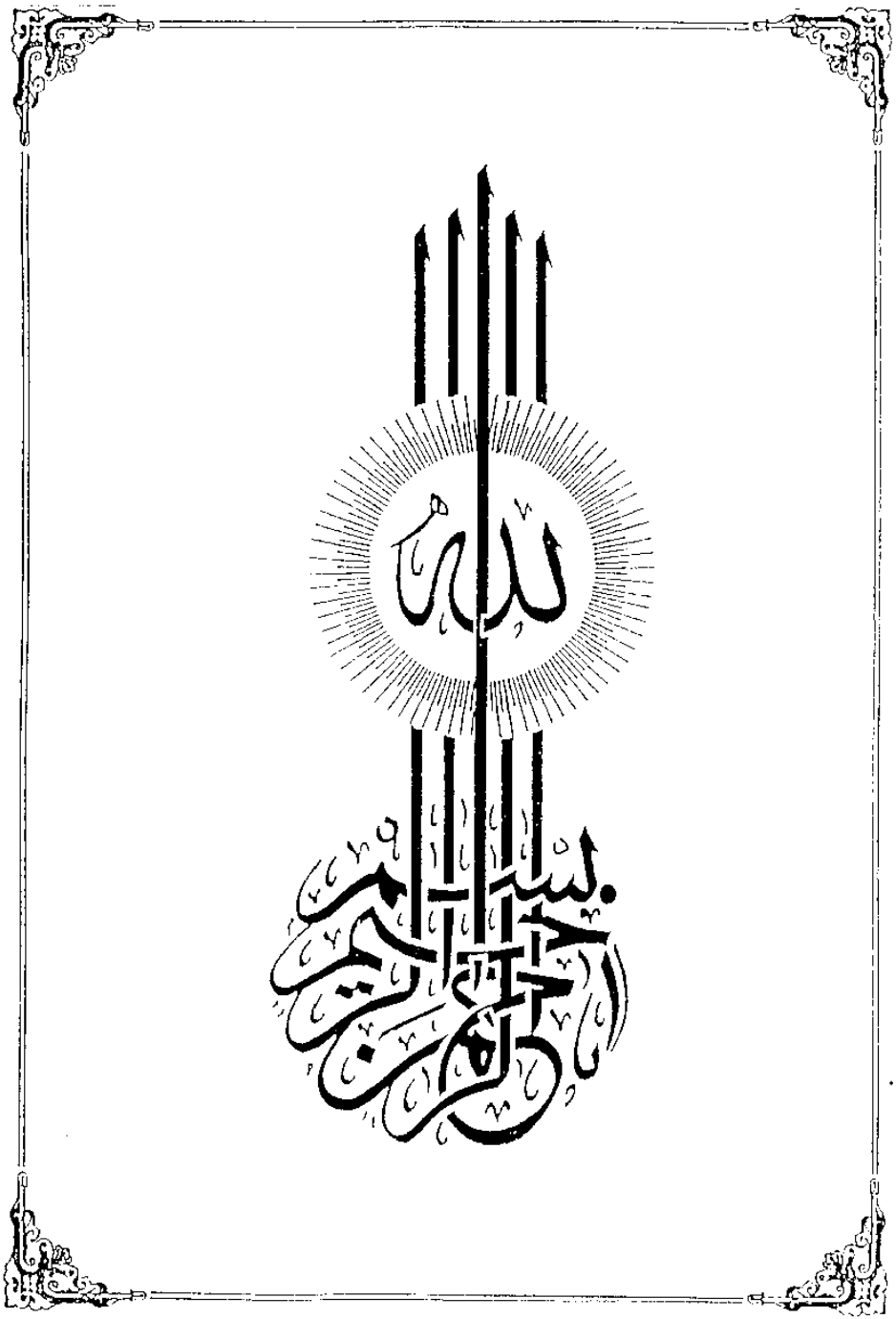

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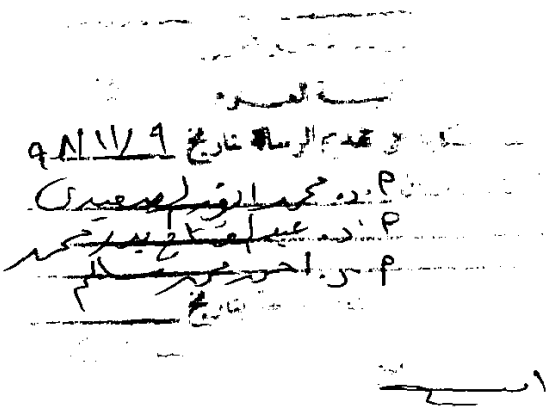
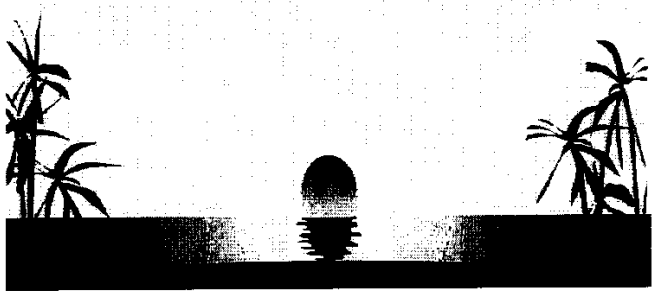


I declare that this thesis has been composed by myself and that the work of which it is a rrecord has been done by myself. It has not been submitted for a degree at this or any other university.

Rania M. Abou Ali

To my parents

and my small family



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Abstract

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Expression of chitinase gene in *E. coli*
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Recombinant pEICHIB plasmid containing chitinase gene (1.7 Kb) was used to transform *E. coli* cells. The chitinase enzyme (54 KDa) was induced with chitin in *E. coli*. Enzyme production increased during the incubation period and reached maximum in 5 to 6 days old culture.

Chitinase enzyme was found to be inducible by chitin and repressed by glucose (1.0%), lactose (1.0%) and mannose (1.0%). The production of chitinase enzyme was directly proportional with chitin concentrations from 0.3 to 1.0% (w/v). After large scale chitinase production, the enzyme was purified using ammonium sulfate precipitation and adsorption-digestion on chitin. Rabbit polyclonal antibodies, have been raised against the 54 KDa purified chitinase, and used for immunodetection of the chitinase activity of 54 KDa protein by Western analysis.

Bioassays were carried out to test the role of chitinase against root-knot nematode *Meloidogyne incognita* eggs biocontrol. It was found that chitinase affected the shape of *M. incognita* eggs and decreased the movements of juveniles. Chitinase also caused early egg hatching and released immature juveniles which died and the life cycle was not completed.

Key words: chitinase- chitin- root knot nematode- *Meloidogyne incognita*.

PAGE	Polyacrylamide gel electrophoresis
PMSF	Phenyl methyl sulfonyl fluoride
rpm	Revolution per minute
SDS	Sodium dodecyl sulfate
TBS	Tris-buffered saline
TBST	Tris-buffered saline + Tween 20
TEMED	N,N,N',N' Tetramethylethylenediamine