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Novel Edible Coating for Tropical Fruits as an Alternative to Synthetic Fungicide

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Edible coating

The potential of an edible coatings to maintain the quality and extend shelf-life of fresh fruits and vegetables , and prevents microbial storage, which is extremely important to perishable horticultural commodities

Coatings can extend shelf-life and marketability

- Delay ripening of the climacteric fruits
- Delay color changes
- Reduce weight loss
- Maintain texture
- Reduce decay
- Simple technology
- Environmentally friendly

Materials used as edible coatings

Proteins- soy, milk, corn, wheat, casein

Carbohydrates- cellulose, pectin, starch, gum

Lipids- Waxes and oils- carnauba waxes, vegetables oils

Resin- shellac, wood rosin

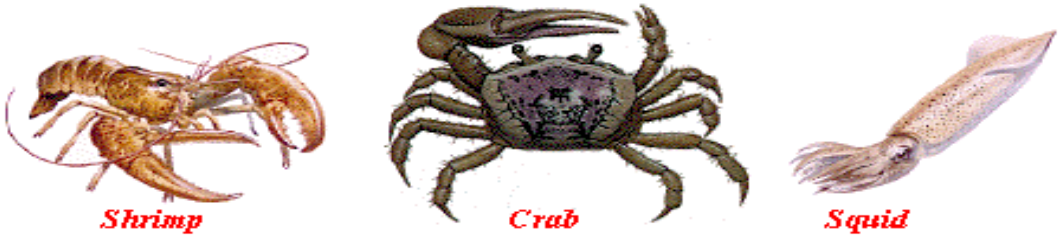
Derivatives of acids and polysaccharides

Semperfresh

Chitosan

What is Chitin/Chitosan?

Chitosan *is a modified carbohydrate polymer derived from the* ***Chitin*** *component of the shells of crustacean, such as crab, shrimp and cuttlefish.*



Shellfish wastes from food processing

↓

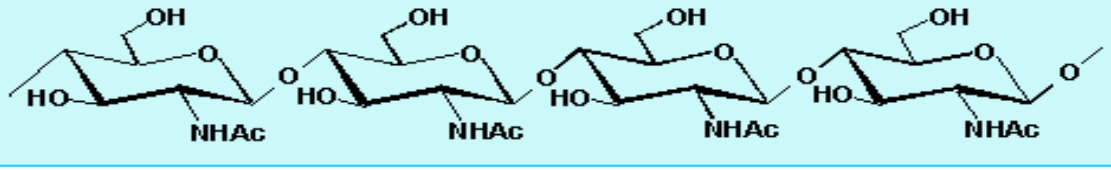
Decalcification *in dilute aqueous HCl solution*

Deproteination *in dilute aqueous NaOH solution*

Decolorization *in 0.5% $KMnO_4$ aq. and Oxalic acid aq. or sunshine*

↓

Chitin



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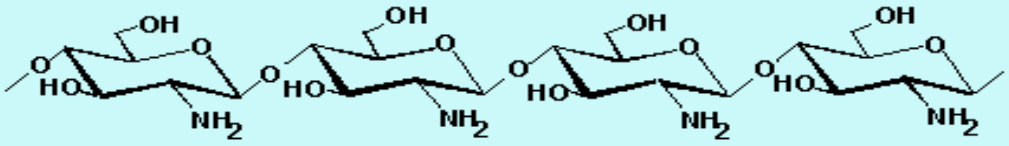
Deacetylation *in hot concentrated NaOH solution (40~50%)*

Chitosan



Prepration of chitin and chitosan

Chitosan powder

- Chitosan is a natural biodegradable compound derived from crustaceous shells such as crabs and shrimps, whose main attributes corresponds to its polycationic nature.

- Chitosan has been proven to control numerous pre and postharvest disease on various diseases on various horticultural commodities.

- It has been reported both soil and foliar plant pathogens fungal, bacterial and viral controlled by chitosan application.

- Microscopical observations indicate that chitosan has a direct effect on morphology of chitosan treated microorganisms reflecting its fungistatic or fungicidal potential.

- Ability to induce resistance by eliciting the activities of antifungal hydrolases and total phenols..

- In addition, chitosan induces structural barriers for example inducing the lignin material for some horticultural and ornamental commodities.

- Ability to form semi-permeable coating, chitosan extend the shelf life of treated fruits and vegetables by minimizing the rate of respiration and reducing the water loss.

- As a non toxic, biodegradable, edible and biologically safe material, chitosan has the potential to become a new class of plant protectant assisting towards the goal of sustainable agriculture.

ANTHRACNOSE INCIDENCE, BIOCHEMICAL CHANGES, POSTHARVEST QUALITY AND GAS EXCHANGE OF CHITOSAN–COATED PAPAYA

- Assess the effectiveness of chitosan in controlling postharvest anthracnose on papaya fruit.
- mechanisms involved in controlling anthracnose by chitosan.
- Biochemical changes of Eksotika papaya coated with chitosan.
- Effects of the coating agent on the physico-chemical characteristics of Eksotika papaya-II.
- Storage life of Eksotika papaya by treatment with a chitosan base coating agent.
- Gas exchange characteristics of chitosan-coated Eksotika-II papaya.

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Papaya

SHORT POSTHARVEST-LIFE

. **Water loss**

. **Accelerated softening**

POST-HARVEST DISEASE

• Anthracnose

(Alveraz, 1987)

(Paull,1997)

APPROACHES

• CA/MA

• Low temperature storage

CHALLENGES

•Resistant pathogens

•Health hazards

•Environmental concerns

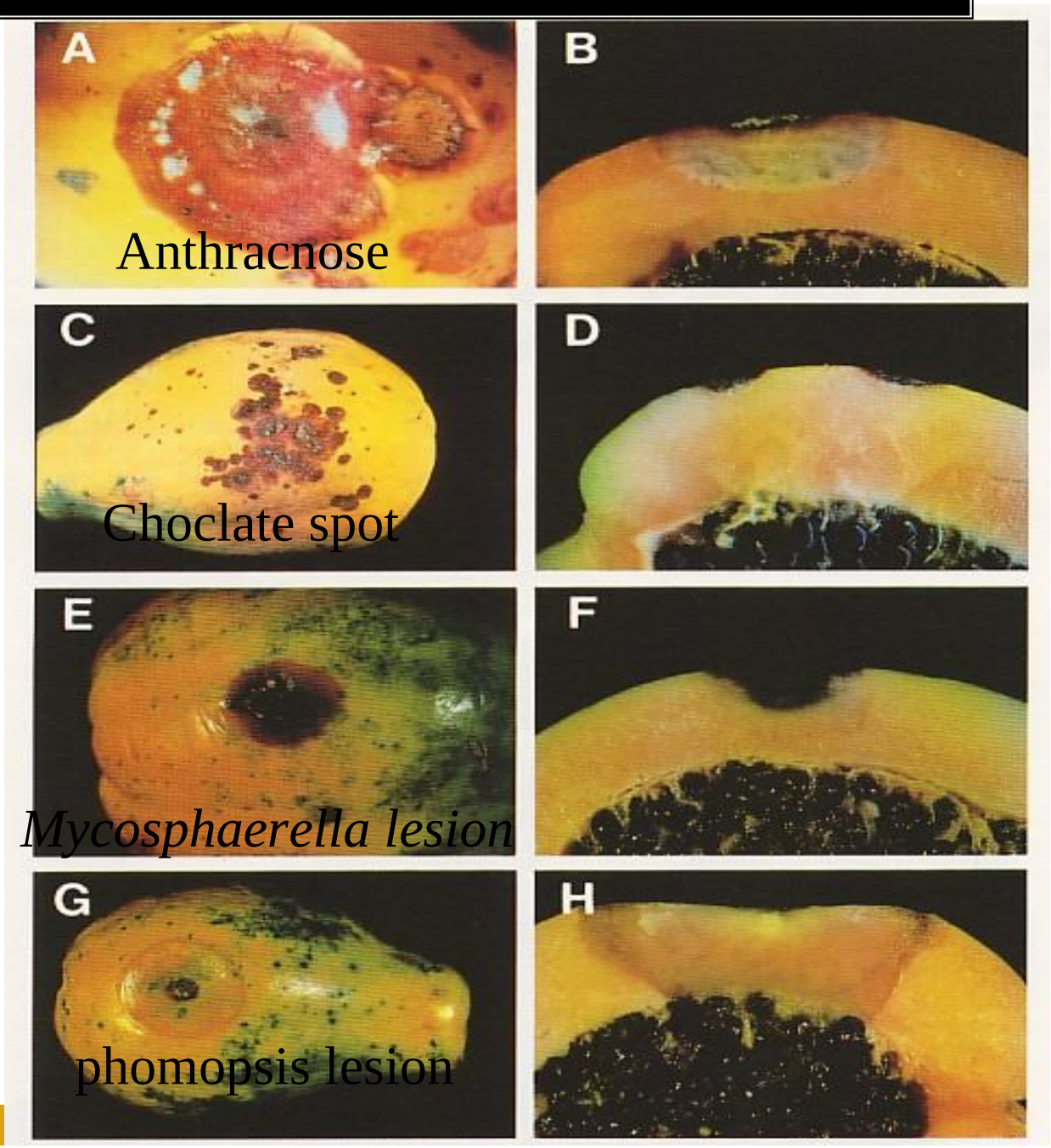
CONTROL

•Chemical fungicides

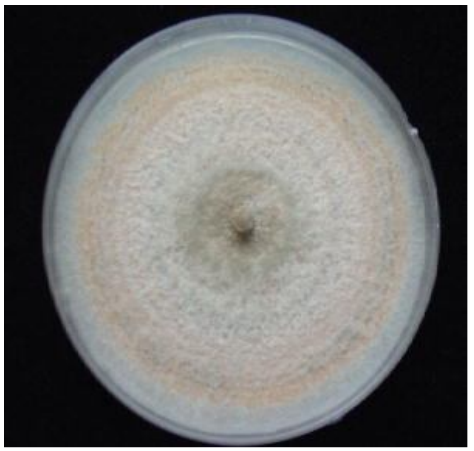
•Hot water dip

Anthracnose, the major postharvest disease of papaya caused by ***C. gloeosporioides***

(Chau and Alvaraz,1987)



Anthracnose Disease of Papaya



Colletotrichum gloeosporioides

1. Effect of chitosan concentration on *in vitro C. gloeosporioides* development

   a. Myceliel growth inhibition

   b. Conidial germination test

   c. Conidial morphology

2. Effect of chitosan concentration on *in vivo* control of *C. gloeosporioides*

   a. Disease incidence

Myceliel growth inhibition

ISOLATE CULTURE FROM INFECTION

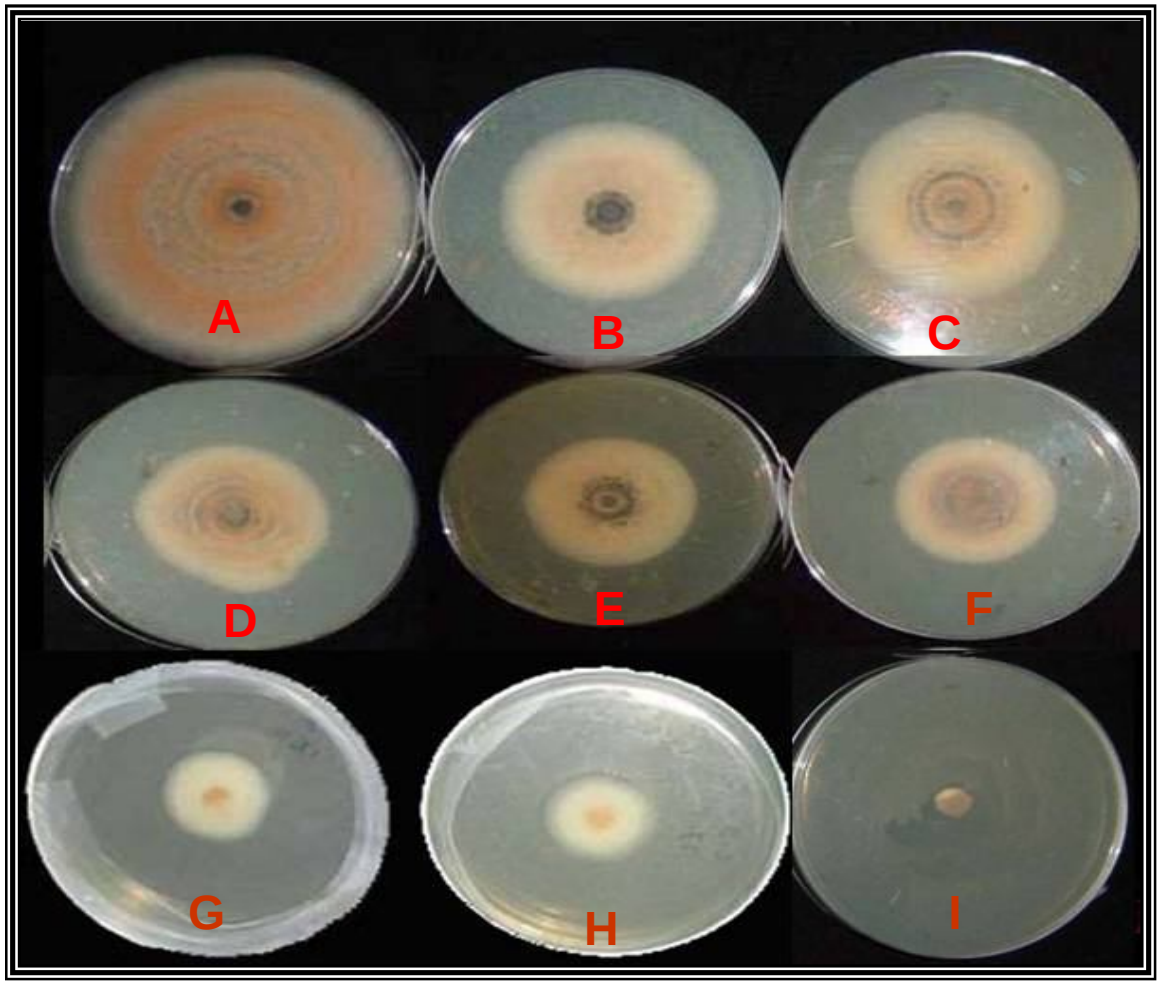
Purify by single spore culture on solid medium (P.D.A)

Prepare medium far control (0+ 0.5% acetic acid) and treatment (0.25, 0.5,0.75, 1, 1.25, 1.5, 1.75 and 2 % (w/v))

Inoculate 1 cm^2 culture from growing tip of pure culture

Observations on growth rate

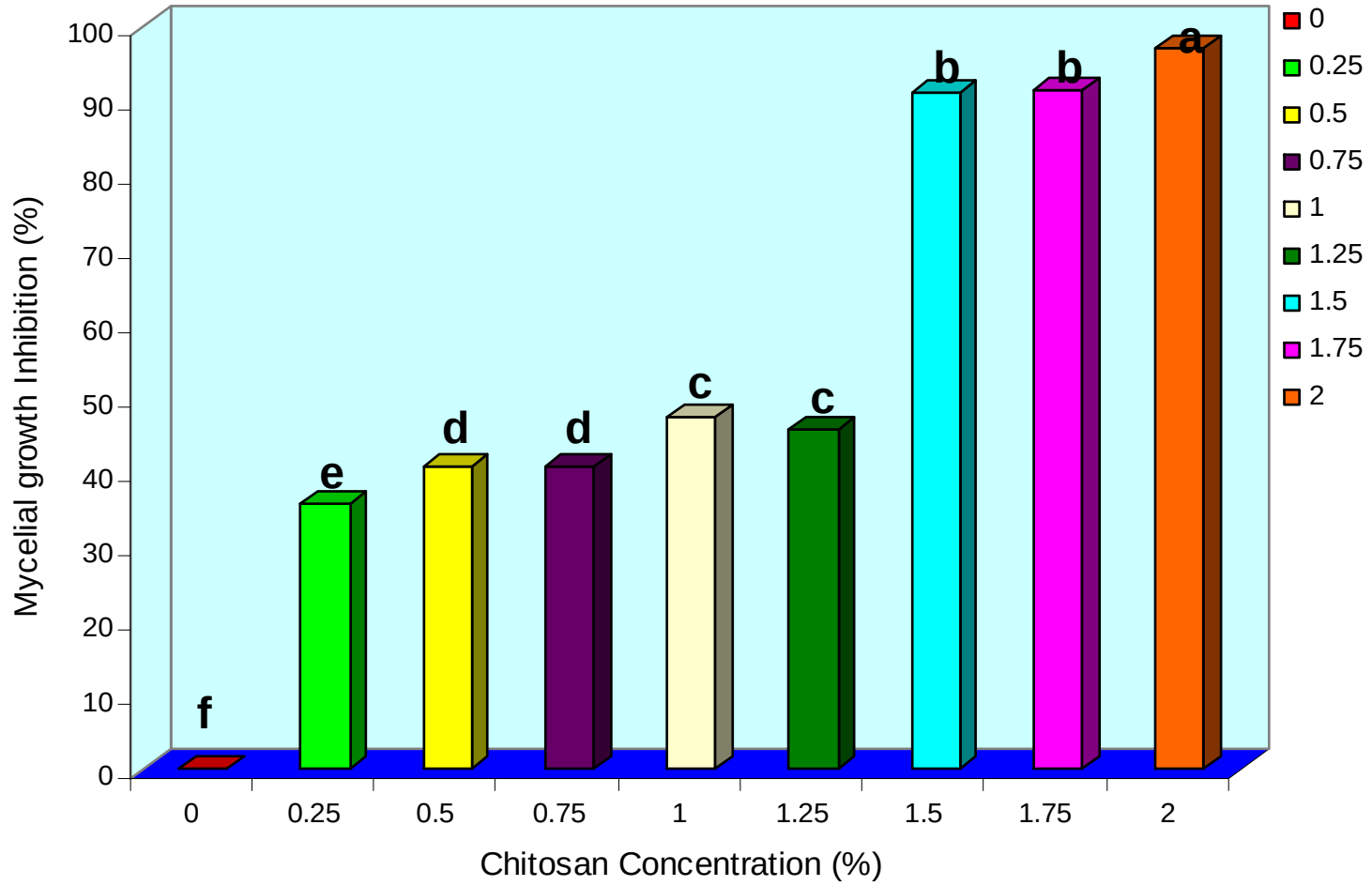
% inhibition in radial growth calculated



A= 0%
B= 0.25%
C= 0.5%
D= 0.75%
E= 1%
F= 1. 25
G=1. 5%
H=1.75%
I = 2%

Effect of chitosan on growth of *C. gloeosporioides* on Potato Dextrose Agar (PDA)

Effect of Different Concentrations of Chitosan on Mycelial Growth Inhibition After 7 days of Incubation at 28 ± 2ºC



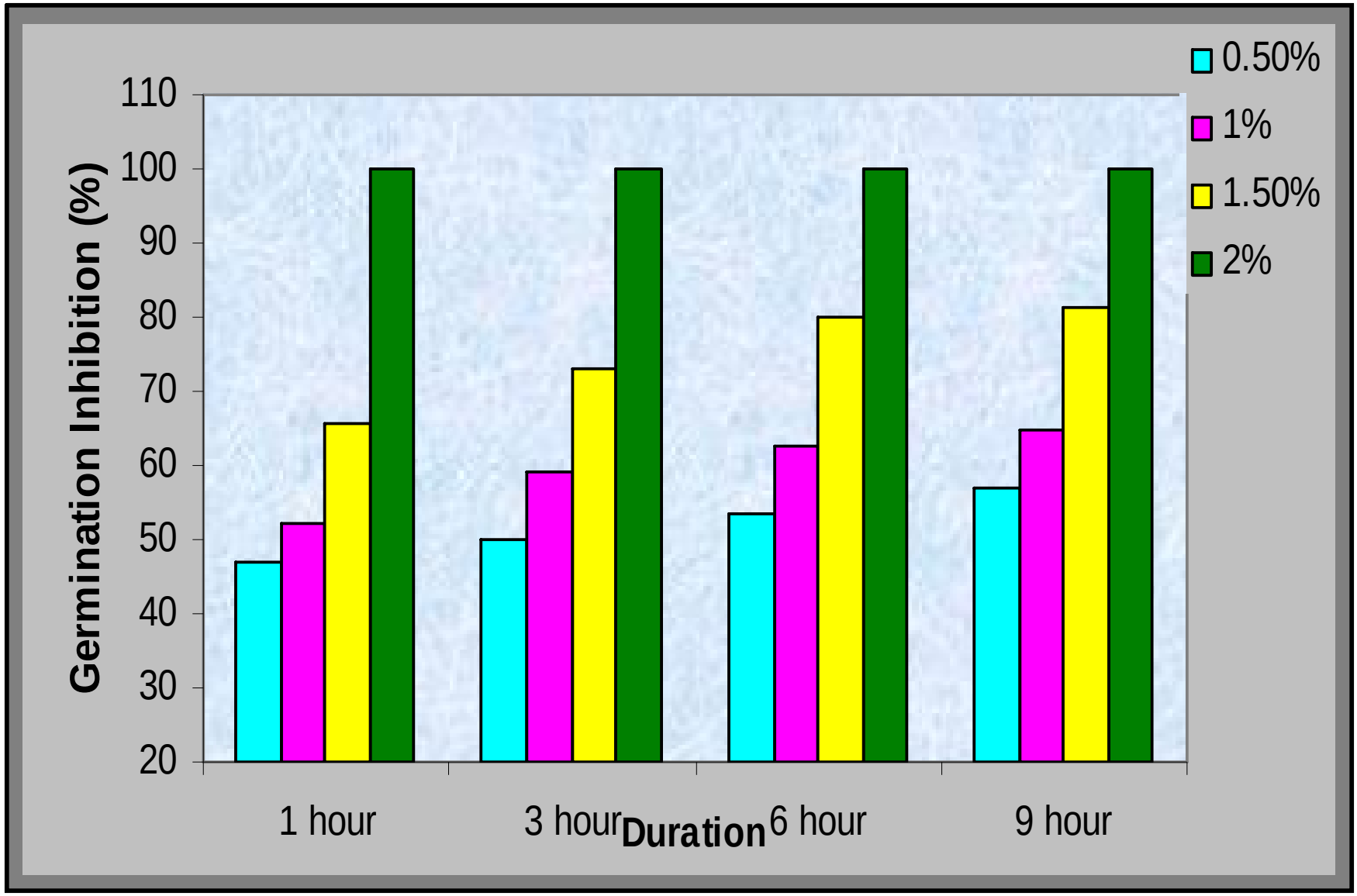
Means with same letters are not significantly different at p ≤ 0.05 using LSD

Conidial Germination

- Cavity Slide Technique
- Germ tube length (Germ tube half the length of conidia)
- 100 conidia / treatment

Microscopic Studies

- Mycelial growth Abnormality
- Conidial germination Abnormality



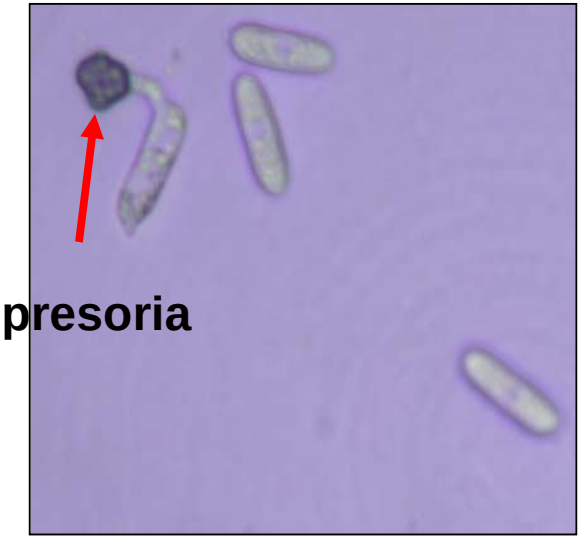
Effect of different concentrations of chitosan solution in on conidial germination of *C. gloeosporoides*

Effect of Different Concentrations of Chitosan on Conidial germination of *C.gloeosporoides* spores after 7 hours of Incubation

Healthy

0 %

0.5 %



Appresoria

1 %

1.5 %

Shrinkage

2 %

Effect of chitosan coating on *in vivo* control of *C. gloeosporioides*

Disease Incidence

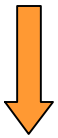
$$DI\ \% = \frac{\text{Number of Infected Fruits}}{\text{Total Number of Fruits Assessed}} \times 100$$

Maturity index of Eksotika Papaya

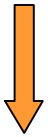
Color index	Skin color
1	Full green
2	Green with trace of yellow
3	More green than yellow
4	More yellow than green
5	Yellow with trace of yellow
6	Fully yellow

Multiply pure culture in liquid medium

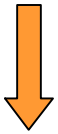
Isolate spores



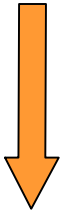
Dilute in sterile water
(Serial dilution)



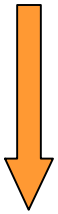
Maintain required concentration (2×10^5 /ml)



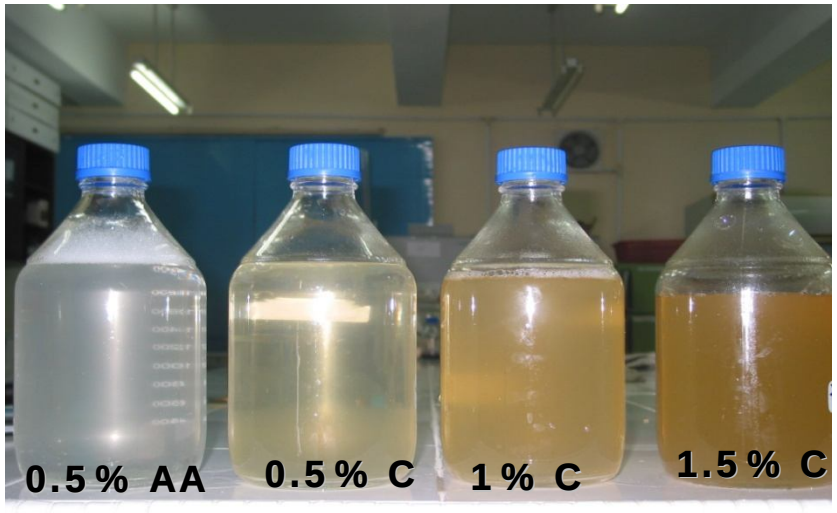
Dip healthy fruit in above concentration (spore) in control



Dip in diff.conc. Of chitosan
(0,0.5, 1, 1.5 and 2% w/v)

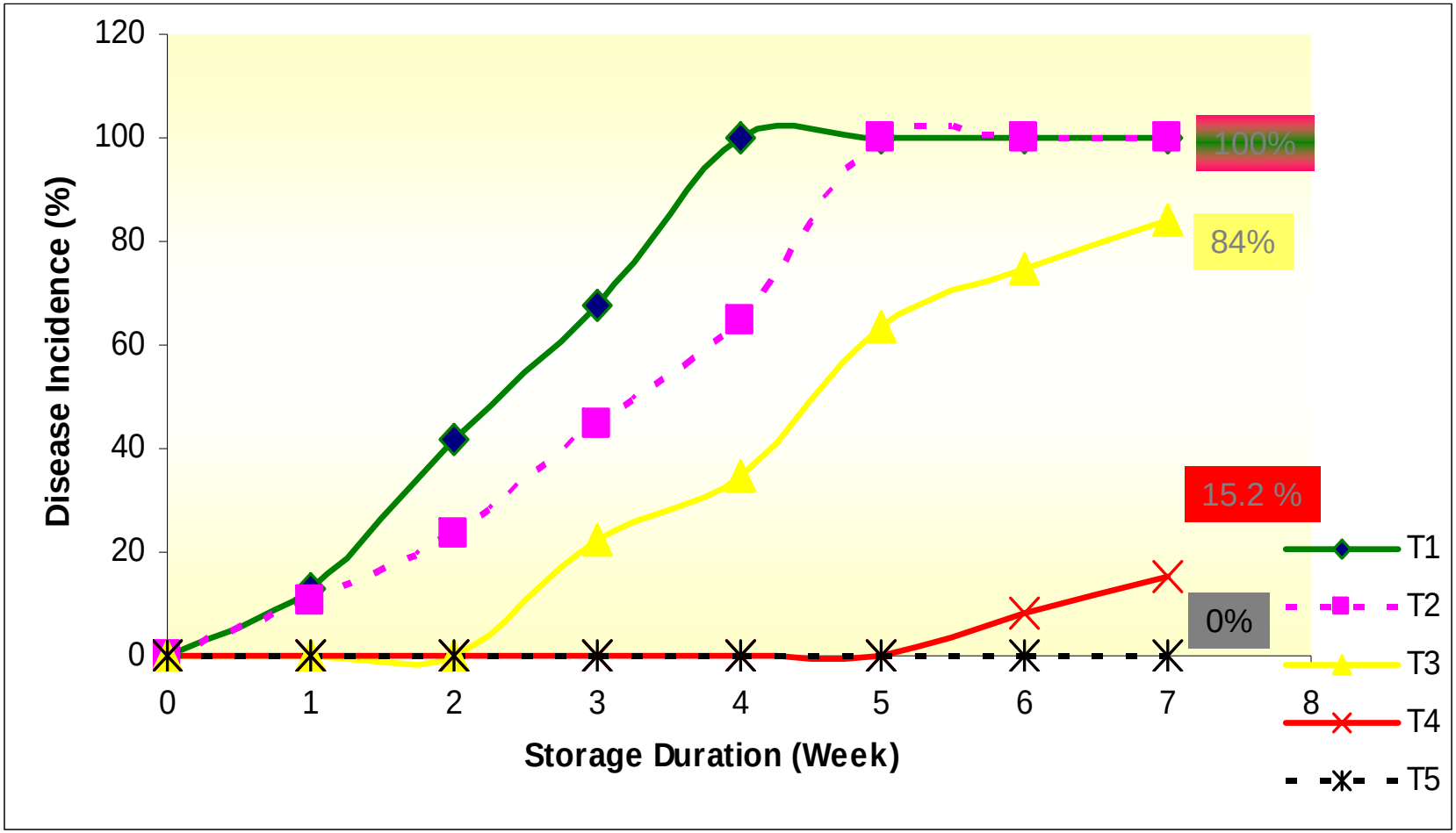


Disease incidence


0.5% AA
0.5% C
1% C
1.5% C


Exotica PAPAYA
FAMA
PRODUCE OF MALAYSIA

Effect of Different Concentrations of Chitosan on Disease Incidence of Anthracnose on Papaya Fruits



T1= 0 %, T2 = 0.5%, T3 = 1%, T4 = 1.5 %, T5 = 2 %

After five weeks of Storage at 12 ^{0} C

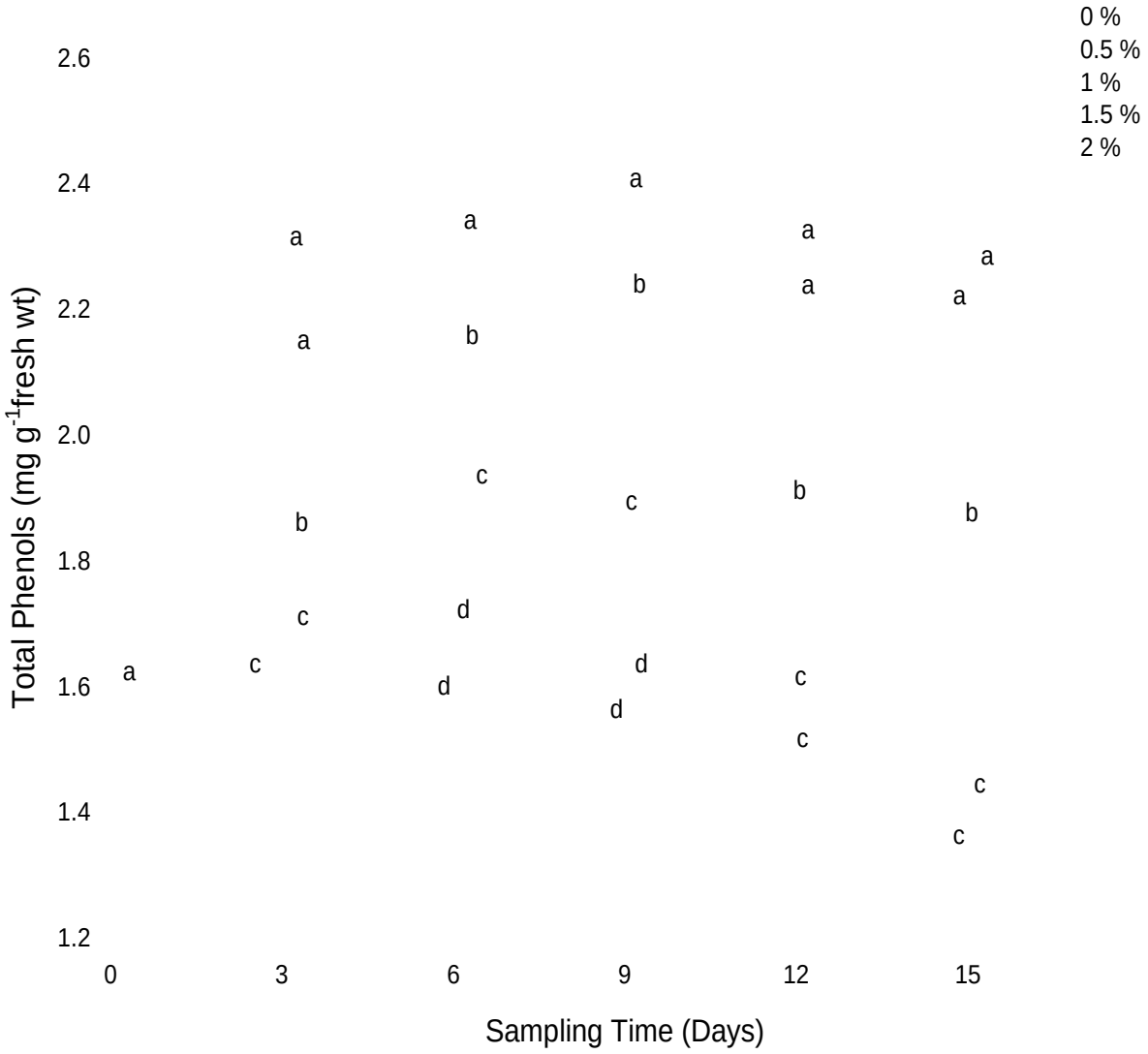
100 % Anthracnose incidence in control

7 % Anthracnose incidence in 1.5% chitosan treated papaya

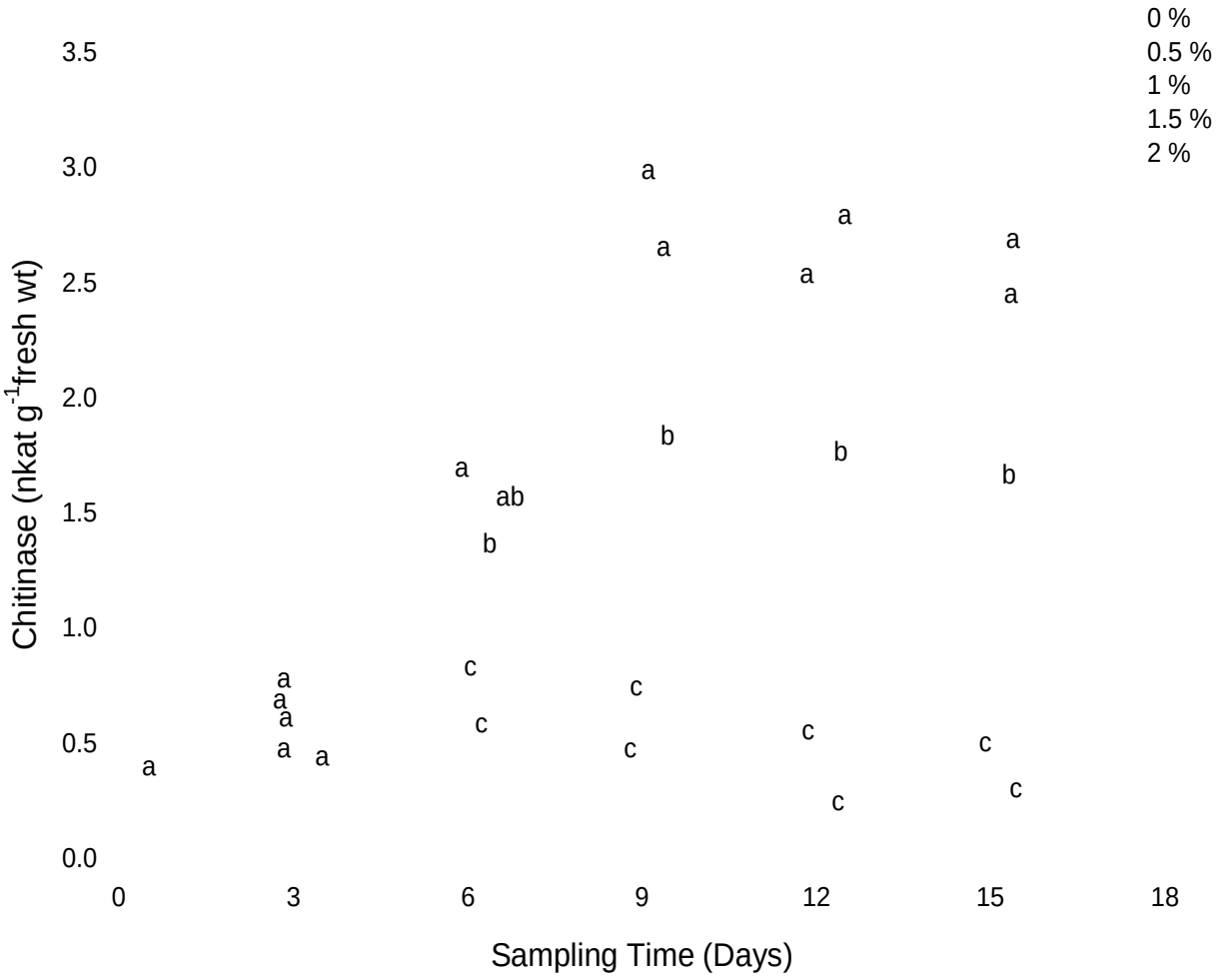
EFFICACY OF CHITOSAN ON THE PRODUCTION OF INDUCIBLE COMPOUNDS IN PAPAYA FRUITS AS INDICATOR OF THE RESISTANCE MECHANISM

Work on natural disease resistance (NDR) has led to a remarkable awareness of the key roles being played by some natural compounds in stimulating the defense response in plants. Elicitors of NDR may be biological, chemical or physical, and may induce local acquired resistance or systemic acquired resistance

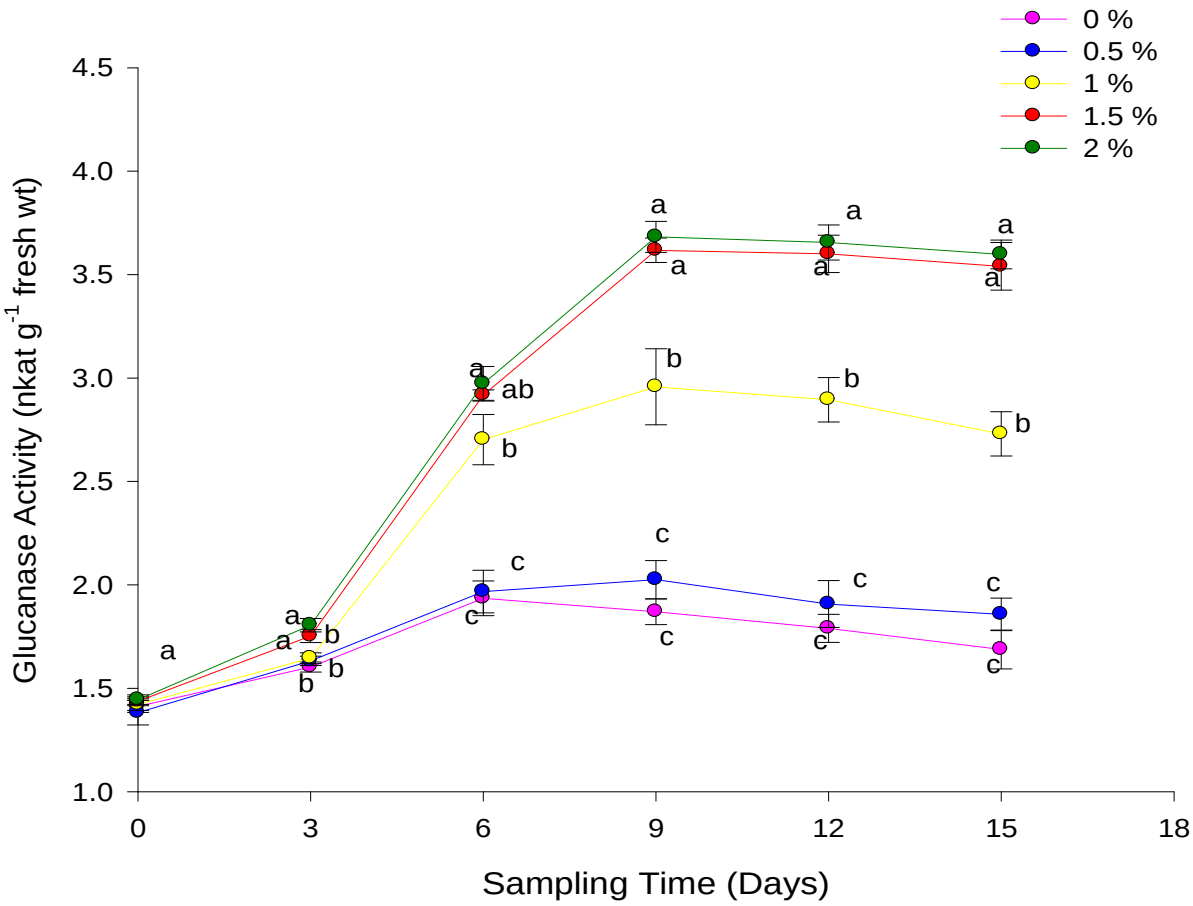
This study was to determine the role of chitosan in the induction of inducible compounds such total phenols,chitinases and β-1,3- glucanases activities in papaya fruits and their modes of action in the suppression of *C. gloeosporioides*.



Effect of different concentrations of chitosan on total phenols during storage



Chitinase activity in papaya fruits treated with different concentrations of chitosan and challenge inoculated with *C. gloeosporioides.* Means with same letters within same week are not significantly different at P ≤ 0.05 using LSD



Glucanase activity in papaya fruits treated with different concentrations of chitosan and challenge inoculated with *C. gloeosporioides*. Means with same letters within same week are not significantly different at P ≤ 0.05 using LSD

Effect of Chitosan Coating on The Physico Chemical Properties of Coated Papaya During Storage

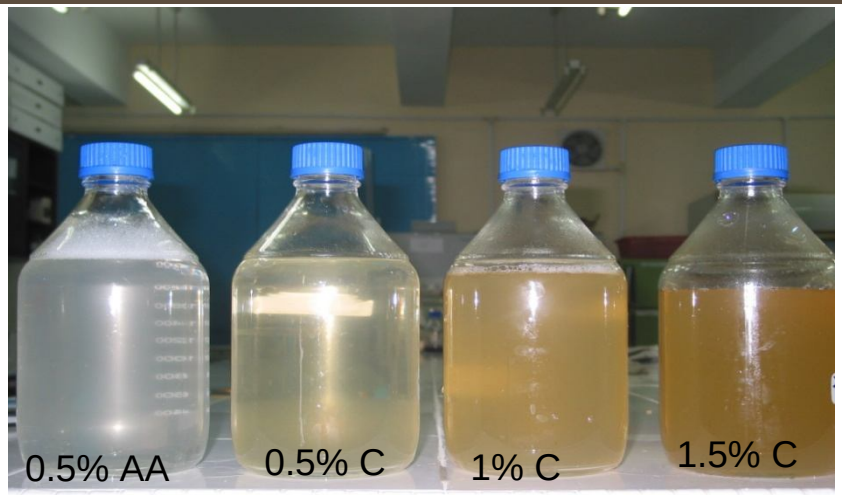
To determine the effects of coating with Chitosan based agent on the physico-chemical characteristics of the coated fruits after and during storage

Chitosan was dissolved in 0.5% acetic acid and 0.1% tween 80 was added for wettebility. The pH of solution was adjusted to 5.6 by adding 2N NaOH .

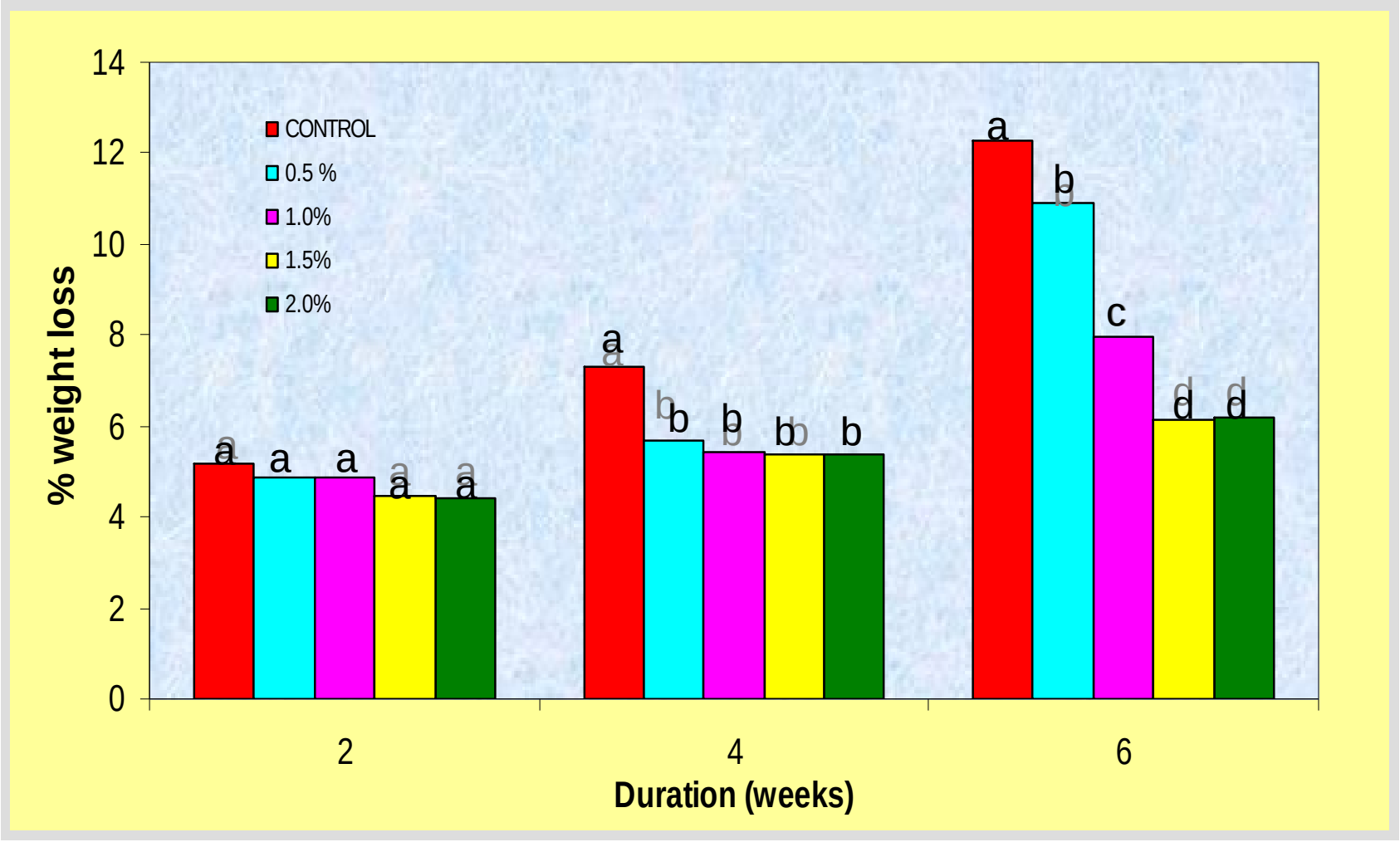
Treatments:

T1 = control
T2 = 0.5% Chitosan
T3 = 1% Chitosan
T4 = 1.5% Chitosan
T5 = 2.0% Chitosan

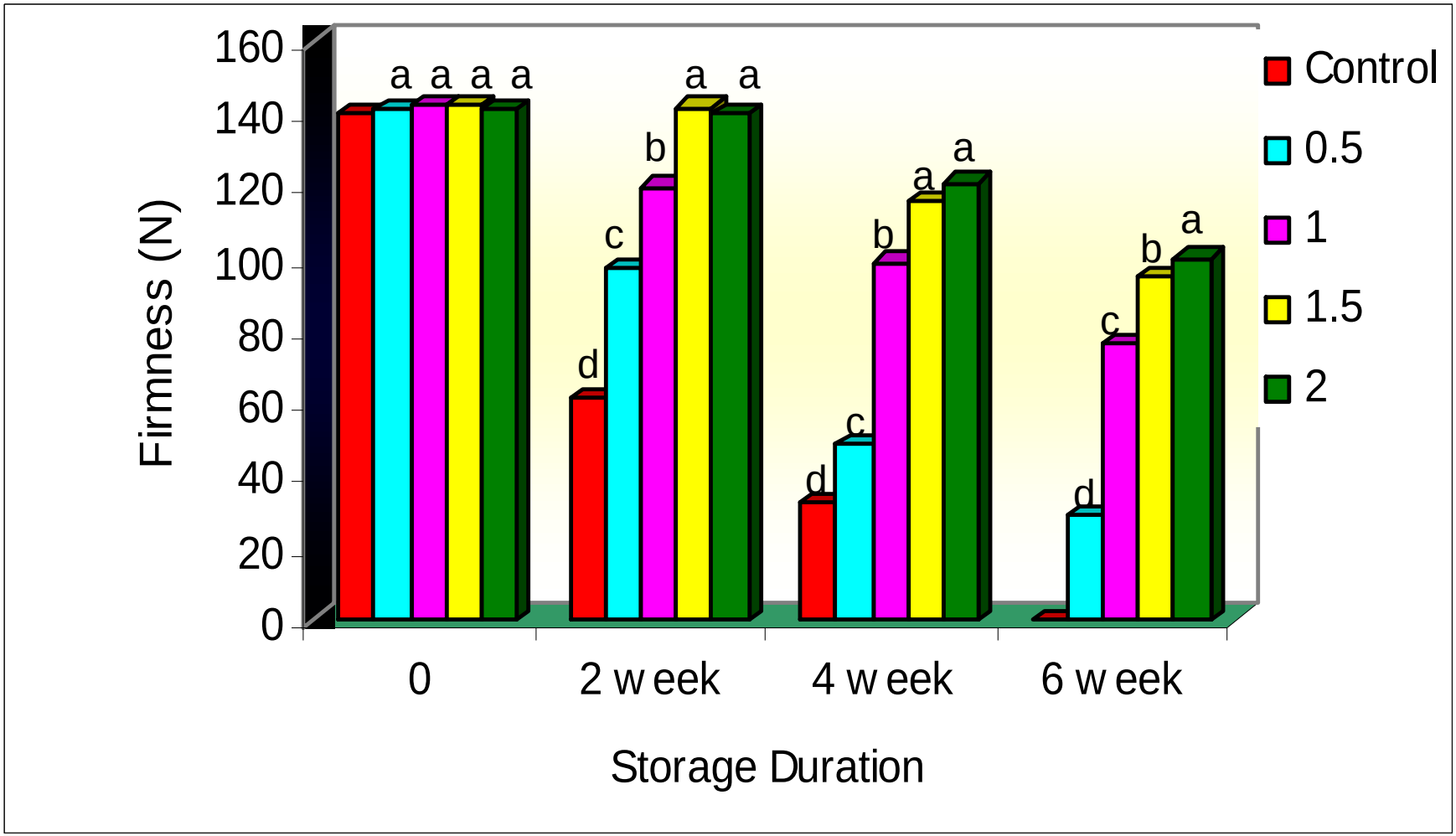
Different Concentrations of Chitosan Solution



Coated Papaya Fruits (1.5%)

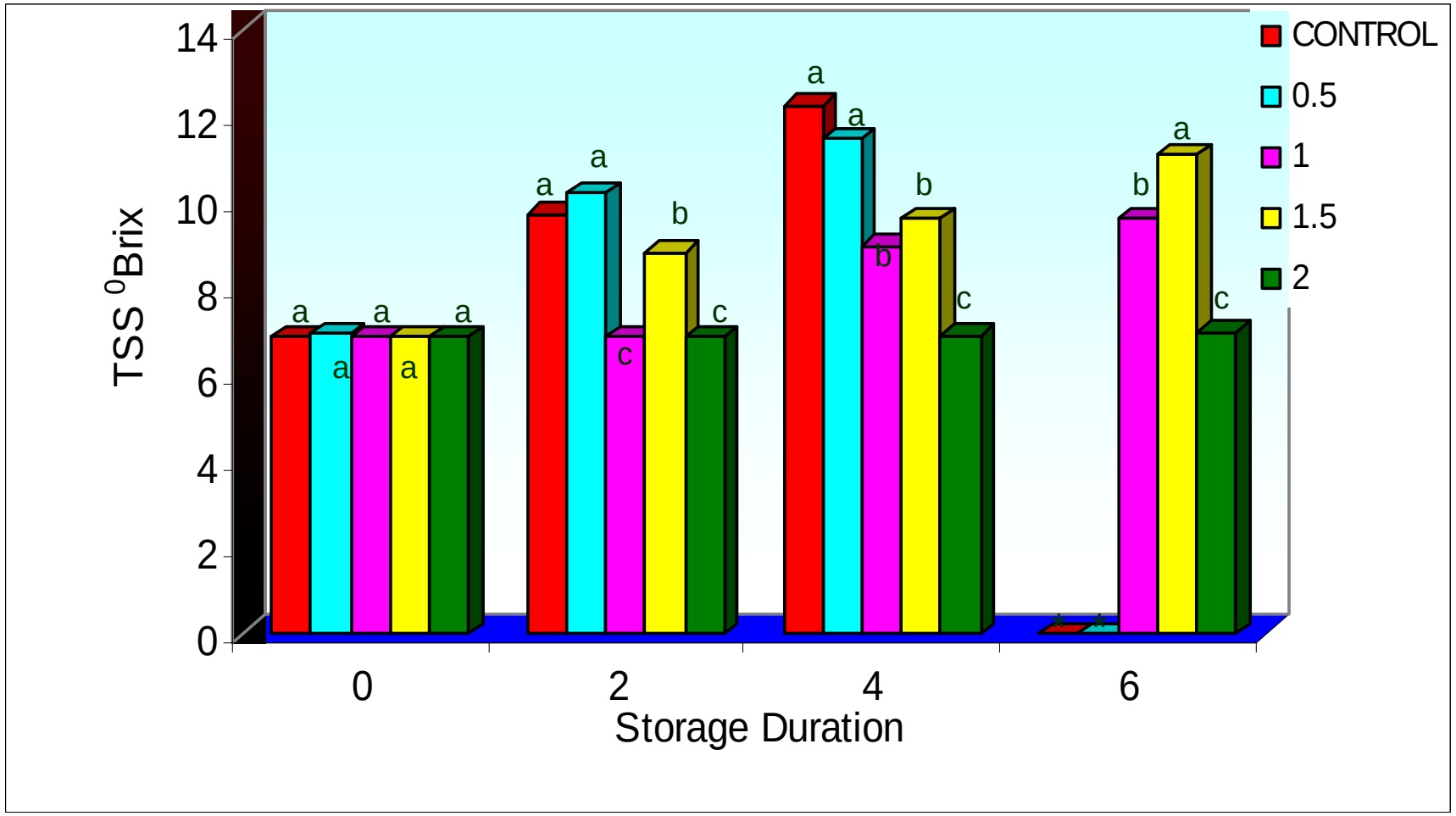


Effect of Chitosan Coating on % Weight Loss in Papaya Fruits During Storage



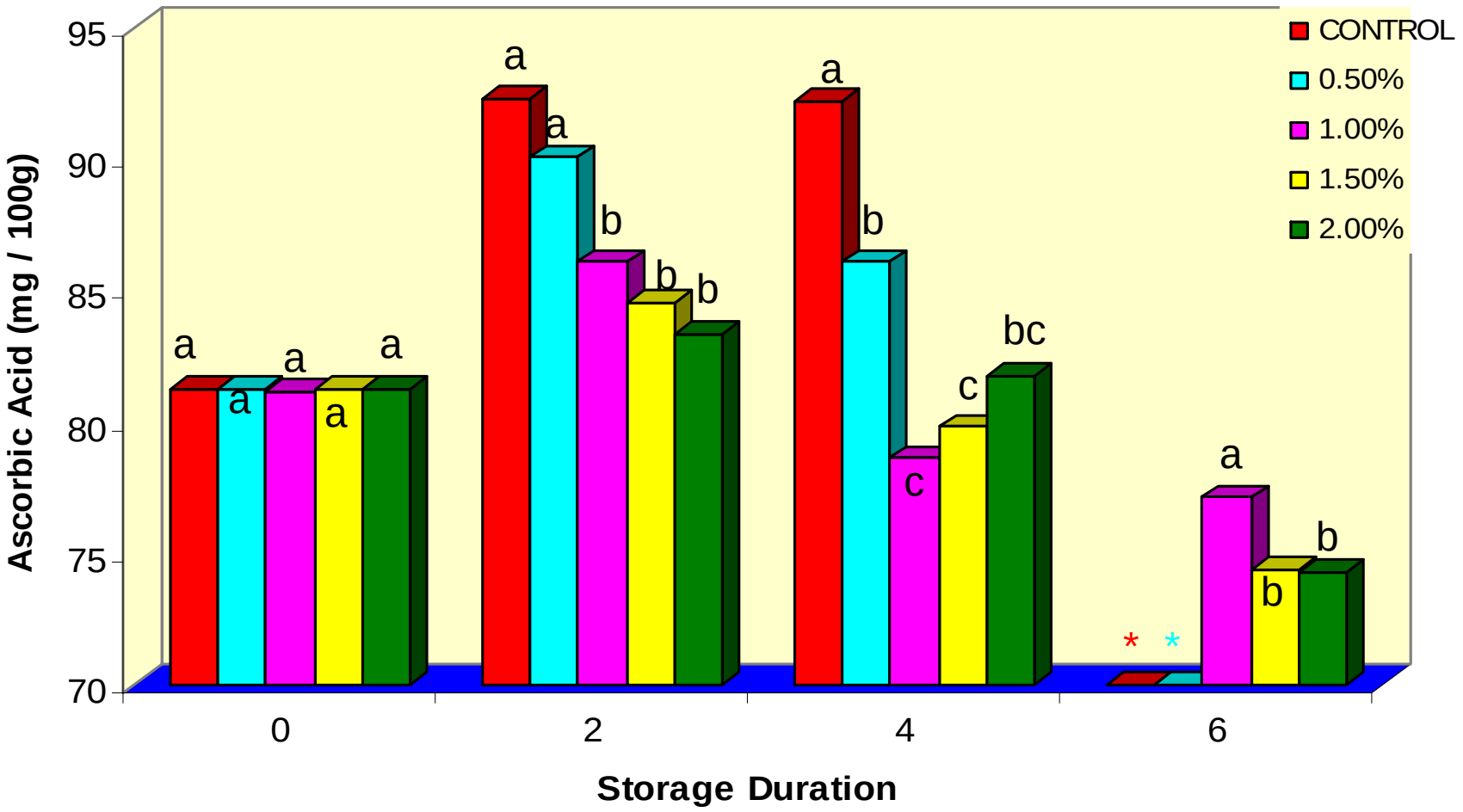
Effect of Chitosan Coating on Firmness of Papaya Fruits

Means with same letters are not significantly different at p≤ 0.05



Effect of Chitosan Coating on Total Soluble Solids in Papaya Fruits up to Six Weeks Duration

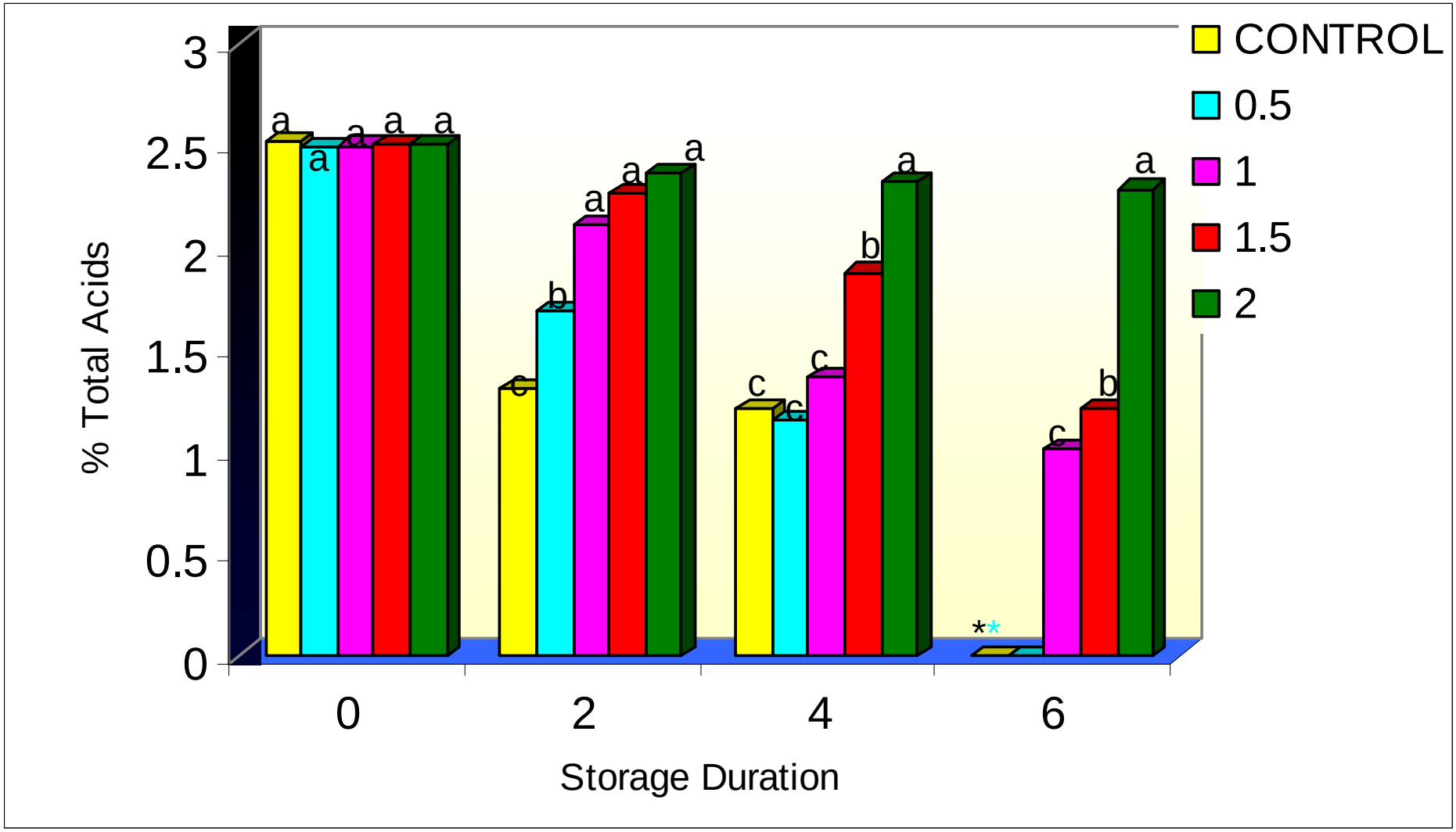
Means with same letters are not significantly different at p≤ 0.05



* * Unacceptable

Effect of Chitosan Coating on Vitamin C in Papaya Fruits up to Six Weeks Duration

Means with same letters are not significantly different at p≤ 0.05



* * Unacceptable

Effect of Chitosan Coating on Total Acid (citric acid) in Papaya Fruits

Means with same letters are not significantly different at p≤ 0.05

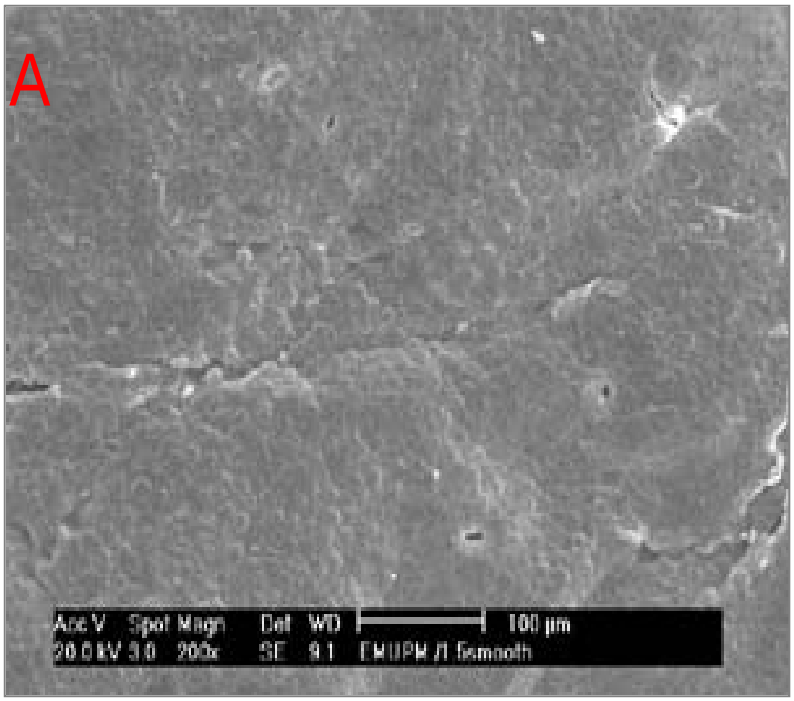
Appearance of papaya fruits after five weeks of storage

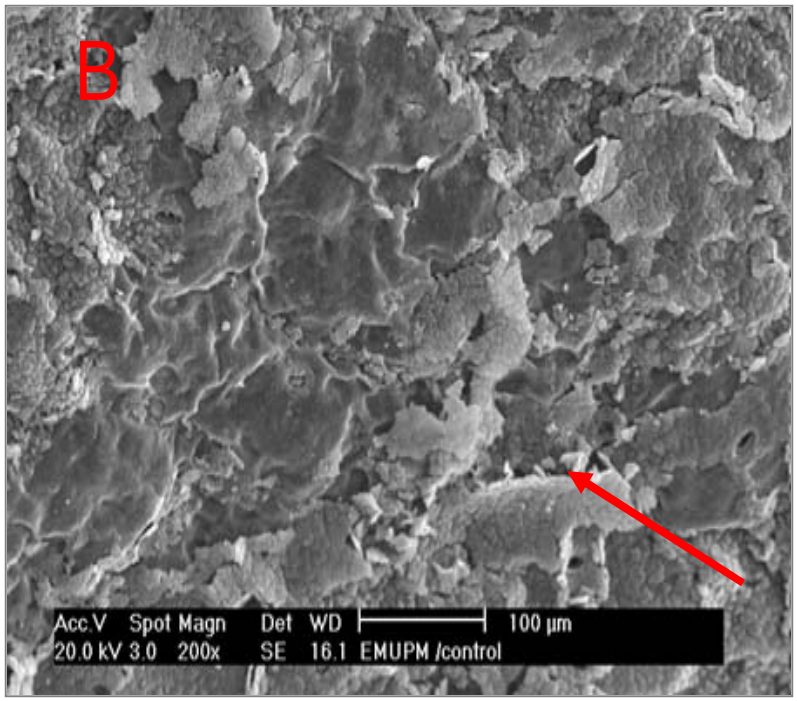
A=control ,B=0.5%,C=1%,D=1.5% and E=2%

Quality After Five Weeks of Storage

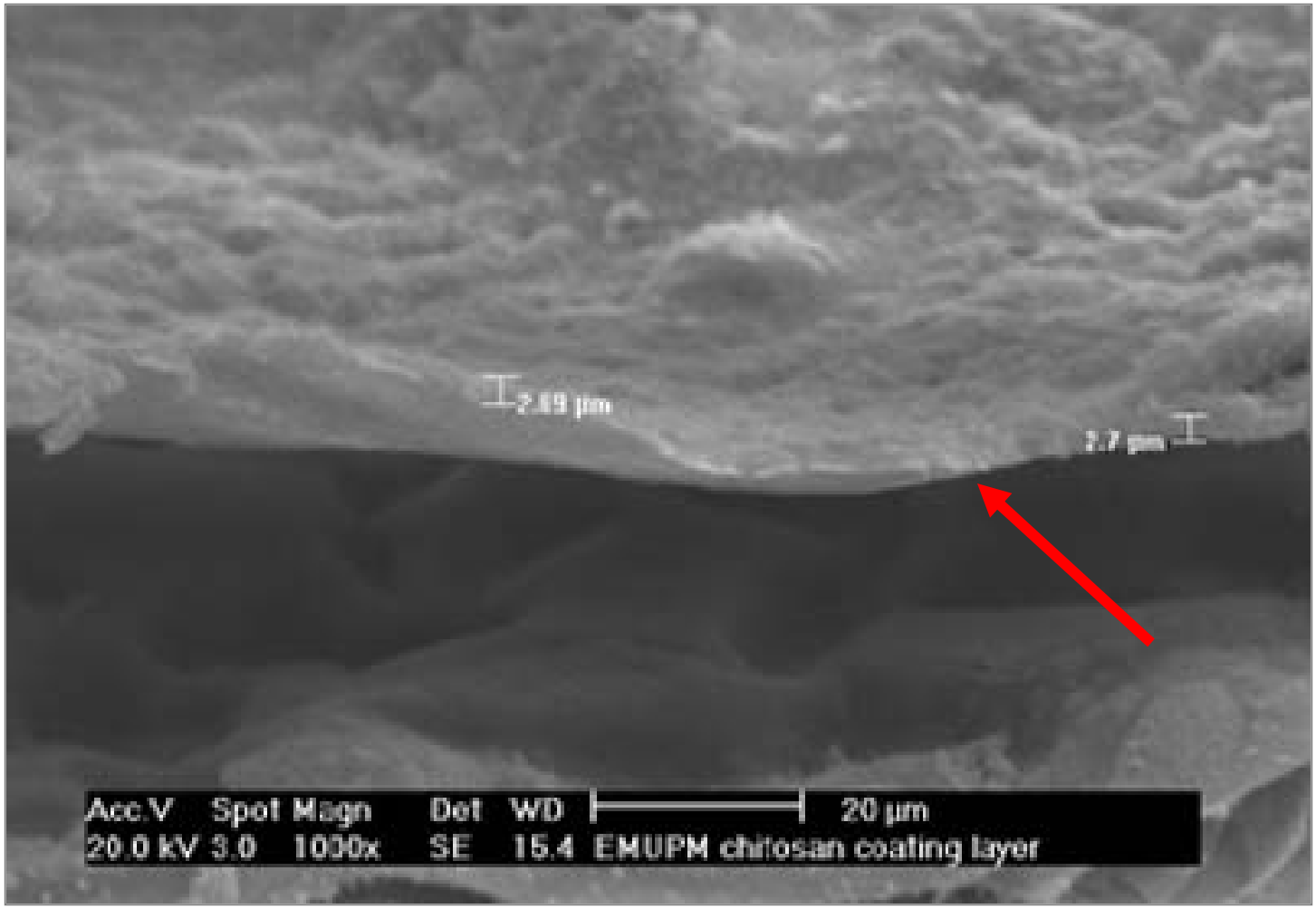
Observation of Papaya surface under SEM

Scanning electron microscope (SEM) study





Scanning electron micrograph (SEMs) of pericarp surface of Papaya fruit. (A) show the surface of 1.5% Chitosan coated; noted Chitosan covered Overall pericarp surface (B) show surface of control fruits; Deep cracks on the skin (Arrow)



Scanning electron micrograph (SEM) showing thickness of the chitosan film (Arrow)

Determination of modified atmosphere of Coated Papaya Fruits

Objectives

- To determine the respiration rate in papaya fruit
- To determine the internal atmosphere in the cavity of papaya fruit

Respiration Rate

Fruits kept in 1 liter air tight jars, sealed
And incubated for 2 hrs at 10º C

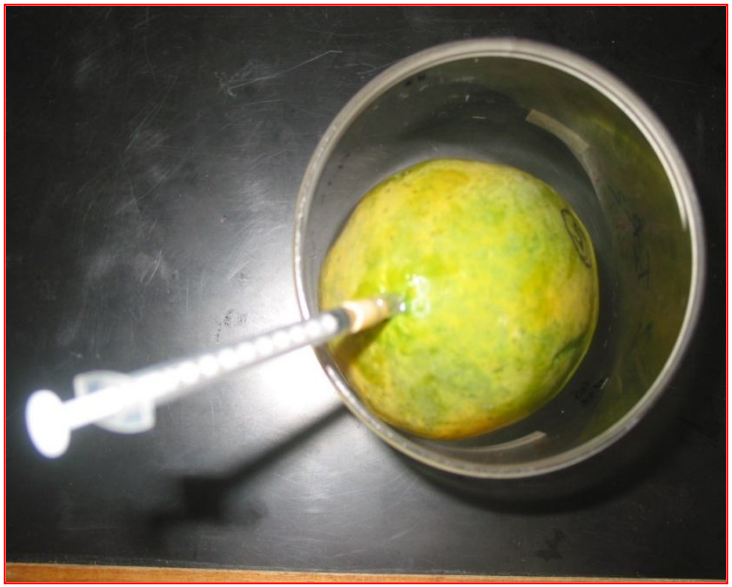
1 ml gas sample with drawn from jars using 1ml Gas- tight syringe

Gas obtained was injected in to Perkin Elmer thermal conductivity detector gas chromatograph equipped with Porapak R (80/100 mesh), using helium as carrier gas.

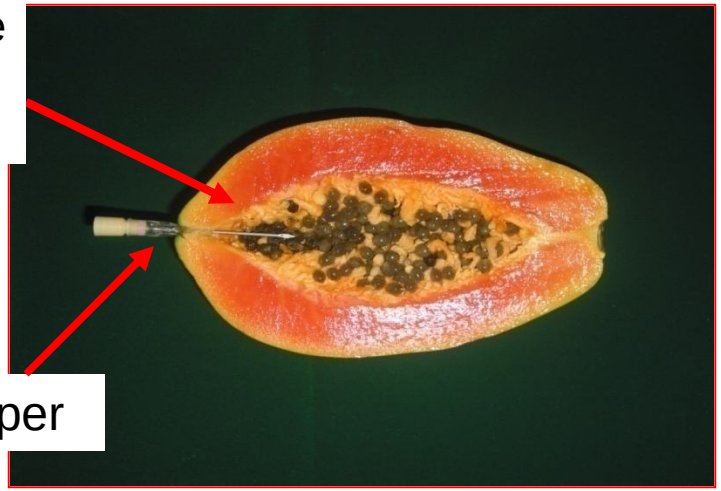
Fruits Incubated for 2 hrs at 10º C

Determination of the internal atmosphere in the cavity of papaya fruit

[redacted]



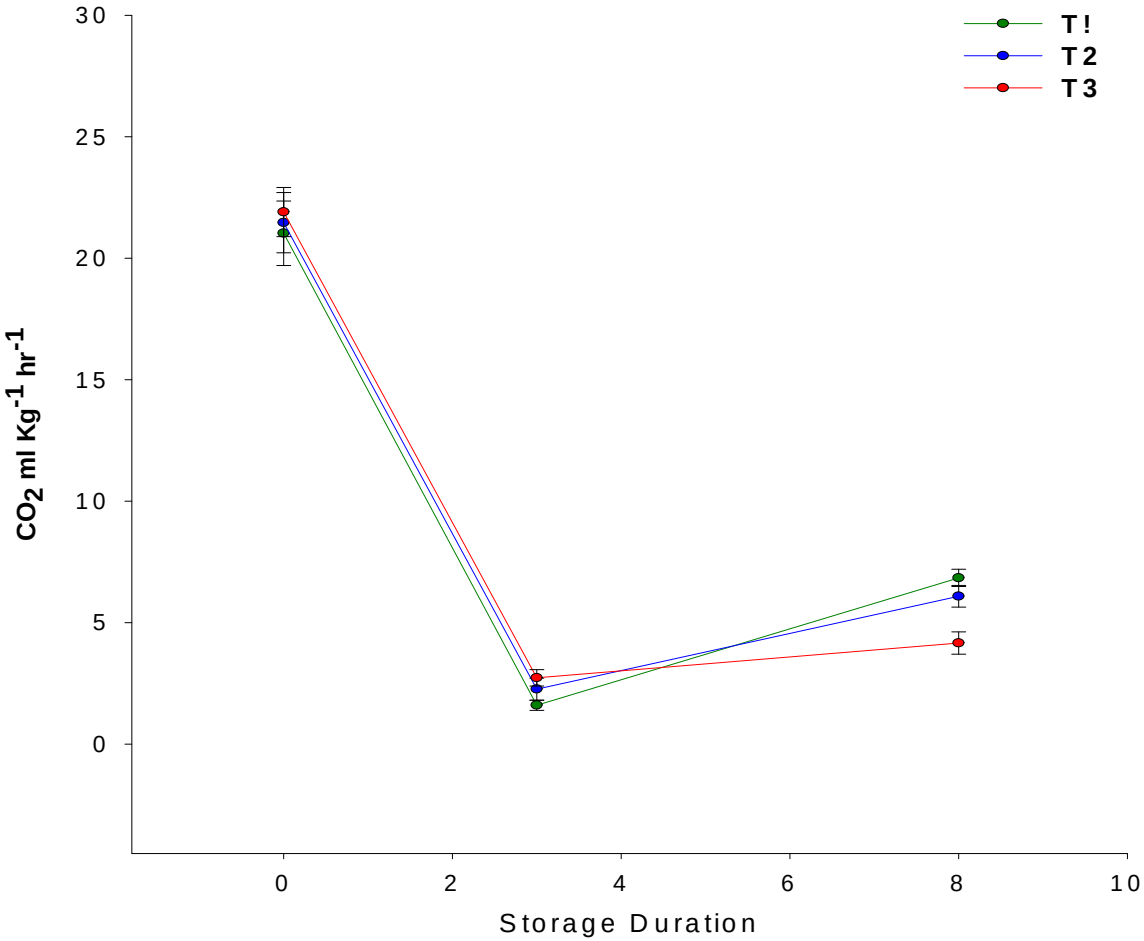
18- Gauge Hypodermic Needle



Hypodermic syringe needle inserted into the cavity of papaya fruit for removal of internal gas sample

Sampling of Gas from Internal Cavity of Papaya

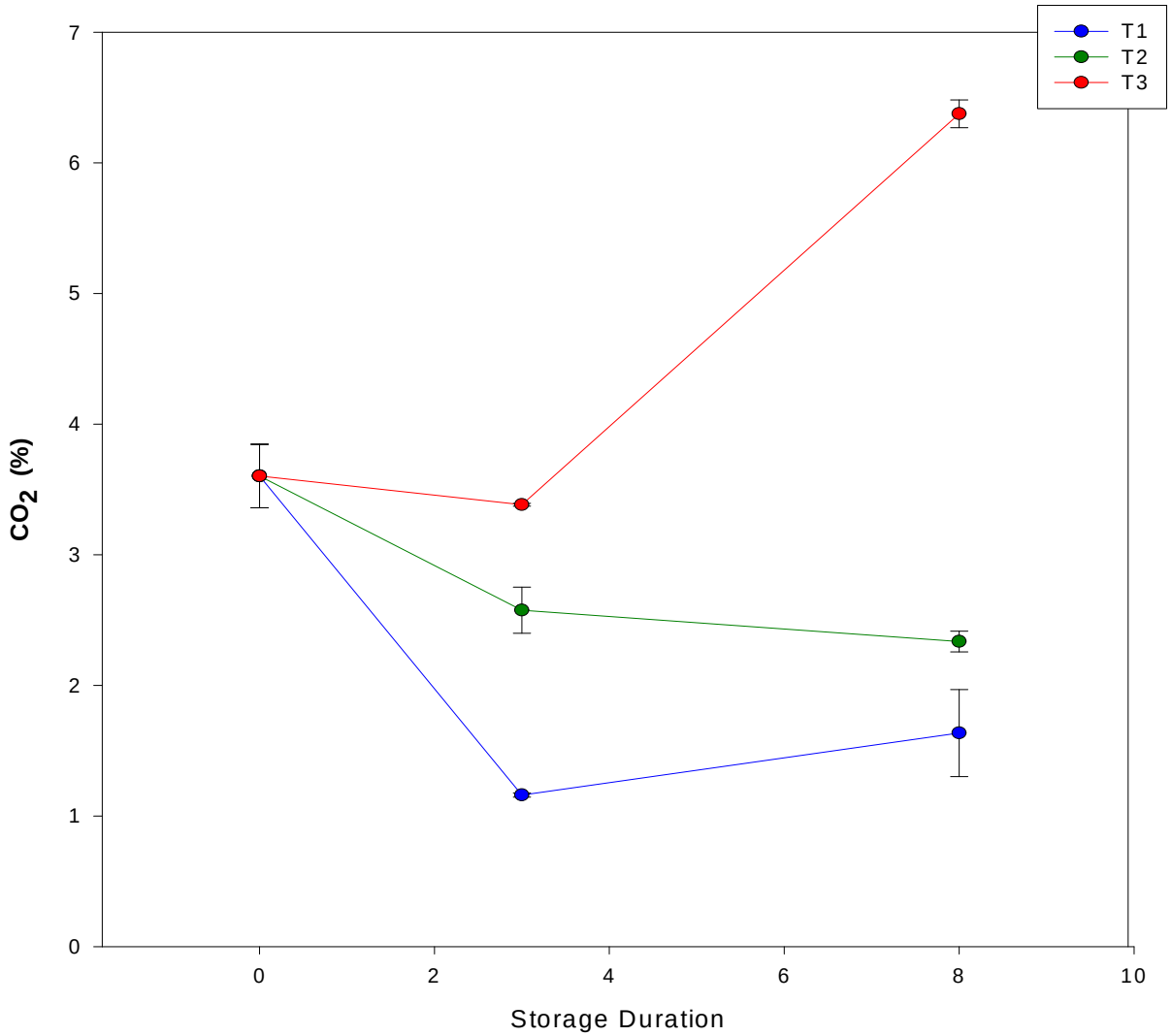
Injection of Sampled Gas in Gas chromatograph



T1 = 0 % (Control)
T2 = 1 % Chitosan
T3 = 1.5 % Chitosan

Respiration Rate of Coated an uncoated papaya Fruits during storage

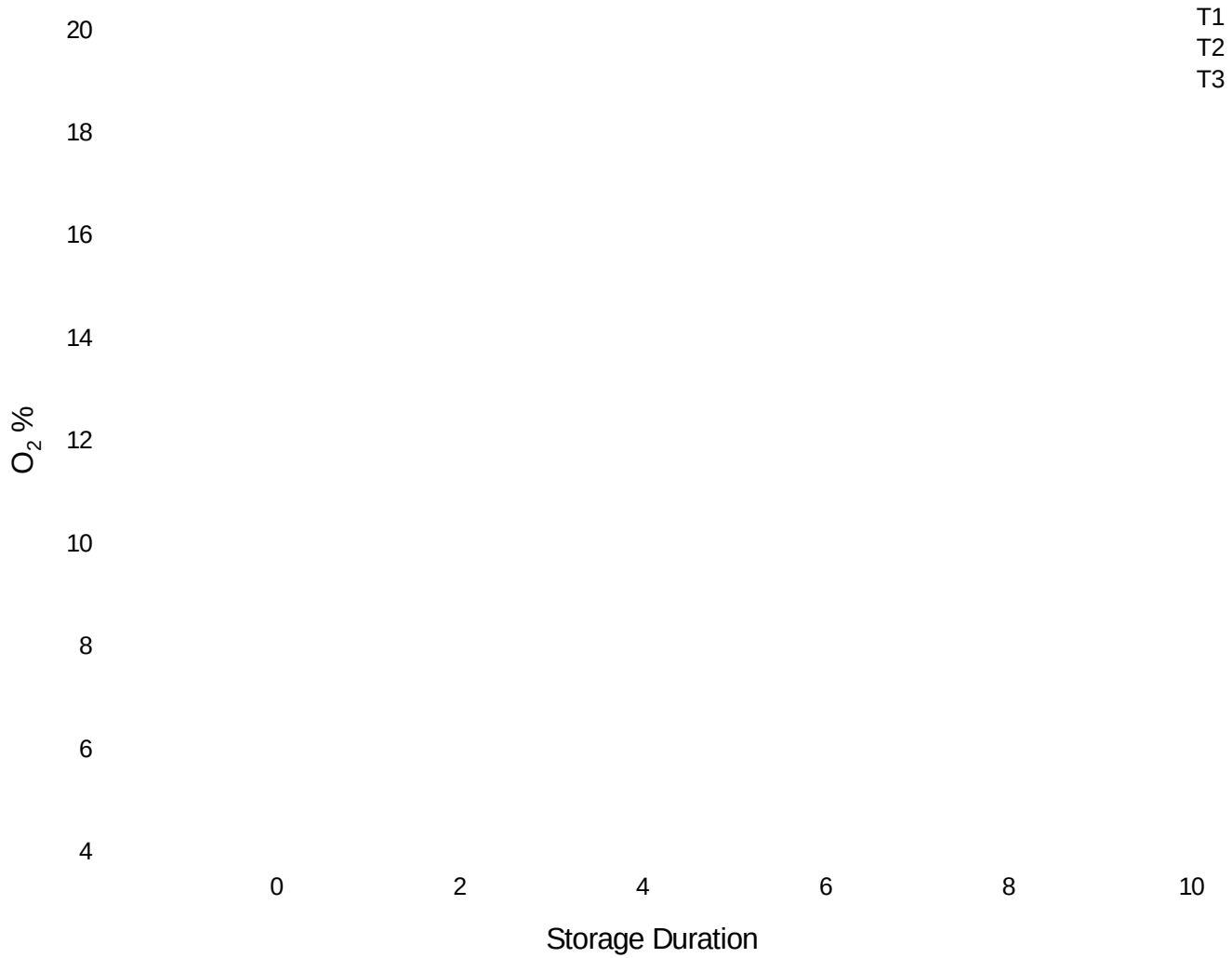
Gaseous Atmosphere from the cavity of Papaya Fruit



T1 = 0 % (Control)
T2 = 1 % Chitosan
T3 = 1.5 % Chitosan

CO_2 % in the Cavity of Coated and uncoated papaya fruits

O2 concentration in the cavity



T1 = 0 % (Control)
T2 = 1 % Chitosan
T3 = 1.5 % Chitosan

O_2 % in the Cavity of Coated and uncoated papaya fruits

Conclusion

- Chitosan markedly inhibited the growth of *C. gloeosporioides* with greater effect at higher concentration on artificial media

- Maximum sporangial germination inhibition occurred in 2 % followed by 1.5 , 1, and 0.5 %, showing trend higher the concentration ,higher the inhibition of spores. There was no inhibition observed in control.

- Spores treated with 2 and 1.5% chitosan showed shrinkage and shriveling and after 24 hrs started to disintegrate

- Papaya fruit coated with 1.5 % chitosan had significantly lower disease development compared to control. Fruits in control treatment rotten completely during 3-5 weeks of storage time and were not of marketable value.

- It is believe that more than 80% of anthracnose on papaya fruit achieved with chitosan is sufficiently adequate to consider chitosan as a natural product to control anthracnose disease.

- SEMs showed that 1.5% chitosan films covered overall surface of the papaya fruits. The films could be retained well up to the end of the storage. while on the surface of control fruits, deeps cracks were observed.

- 1.5 and 2% chitosan coating significantly retarded the weight loss Of papaya fruits. Chitosan film formed on the surface of the fruit delayed migration of moisture from the fruit into environment

- The total acidity (citric acid) was dependent on chitosan concentration and storage duration. The rate of citric acid decreases was lower with chitosan concentration except 2%

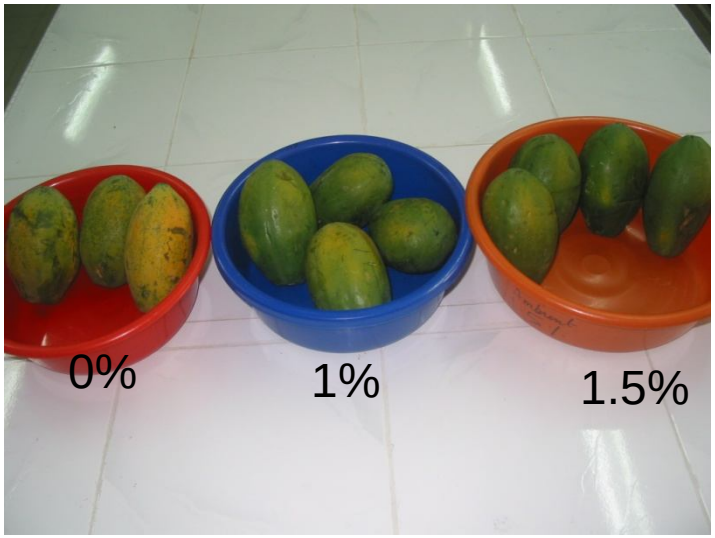
- Carbon dioxide evolution of chitosan coated fruit decreased gradually during storage and was significantly lower in levels than that of non coated fruits

- Chitosan coating also raised the internal CO_2 and decreased the internal O_2 level with in the fruits, the greater effect was found at 1.5%

- Chitosan coating delayed rate of ripening as indicated by the colour of the fruits.

- Chitinase, Glucanase and total phenols activities of papaya fruits increased during storage. It showed that chitosan is able induce resistance against anthracnose disease during storage.

Papaya fruits at Ambient Temperature After 10 days



Prospects

Extension of storage upto five weeks would facilitate the export of fruits to long distance markets by sea and thereby cost of export would be reducing making the fruits more competitive in the world market.

Nutshell

These effects have been attributed to its direct antifungal activity, induction of postharvest resistance responses and creation of modified atmosphere in papaya fruits.

Current research

Project: Screening of antagonistic bacteria to enhance the efficacy of chitosan to control anthracnose disease of papaya.

- Optimization of the chitosan coating during transportation.

Thank you for your attention

Have a fruitful day

Ranked UK TOP 10 and
GLOBAL TOP 70 UNIVERSITY
(SJTU and Times Higher rankings)

Any Queries:
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