

3 **Effect of chitosan biopolymer on microbial decay in tomato (*Solanum***
4 ***lycopersicum L.*)**

5
6 **Abstract**

7 The current study sought to investigate the influence of chitosan biopolymer against microbial
8 decay in tomato. In the present study, bulk-chitosan at different concentrations (0.01, 0.04,
9 0.08, 0.12, 0.16, 0.20% w/v) and Cu-CS NPs (0.01, 0.04, 0.08, 0.12, 0.16, 0.20% w/v) along
10 with control were evaluated on tomato variety 'Dev' by dipping fruits for 6 min and stored
11 at ambient temperature (27°C±2). Bulk-chitosan at 0.16% was found most effective to prevent
12 microbial decay and maintain sensory evaluation from day 1 to days 21 as compared to
13 control. Based on the aforementioned results and comparative evaluation of different doses of
14 bulk-chitosan, we concluded that chitosan is very effective at less concentration and thus
15 exert minimum chemical load on the treated tomatoes. Therefore, it may claim that chitosan
16 biopolymer have potential to protect tomato against microbial decay.

17 **Keywords:** Chitosan, biopolymer, microbial decay, tomato

18
19 **Introduction**

20 Tomato (*Solanum lycopersicum L.*) is the second most important vegetable crop after potato
21 (FAO, 2021), and it is a model plant for researchers, especially for those studying the growth,
22 yield and quality of fleshy fruits (Bertin and Genard, 2018). Tomato contains higher amounts
23 of lycopene, a type of carotenoid with antioxidant properties which is beneficial in reducing
24 the incidence of chronic diseases like cancer and many other cardiovascular disorders
25 (Viskeliš et al., 2015). India is the 2nd largest (11.5%) producer of tomato in world. According
26 to FAOSTAT (2021), tomato was grown world-wide in an area of 5.16 MH with the
27 production of 189.13 MT and productivity of 36.6 MT per hectare. In India it was grown in
28 0.84 MH with the production of 21.18 MT and productivity of 25.06 MT per
29 hectare. Chitosan is a linear amino polysaccharide of glucosamine and N-acetylglucosamine
30 units and obtained by alkaline deacetylation of chitin extracted from the exoskeleton of

31 crustaceans such as shrimps and crabs as well from the cell walls of some fungi (Sucharitha *et*
32 *al.*, 2018). It is being widely used in different fields ranging from medicine, tissue and bone
33 engineering to food sector, cosmetics, textiles, pharmaceutical, biotechnology, paper industry
34 and in waste-water treatment (Jiang and Li, 2005). In agriculture, **chitosan** is an ideal
35 antimicrobial agent in preservation of fruits and vegetables. **Chitosan** is generally used to
36 prevent microbial diseases and decay of fruits and vegetables through its pre- and post-
37 harvest treatments (Bautista-Banos *et al.*, 2006). Its broad-spectrum antibacterial activity was
38 first proposed by Allan. **Chitosan** is considered as a strong antimicrobial agent due to its
39 positive surface charge which alters the microbial membrane structure that eventually leads to
40 leakage of cellular fluids and death (Goyet *et al.*, 2009). The NH₂ group present in it is
41 responsible for its bioactivity against microbial community through inhibition of gene
42 expression and protein synthesis (Kong *et al.*, 2010). Chitosan has been used to prevent post-
43 harvest losses in fruits and vegetables. It is also effective in reducing post-harvest diseases
44 caused by various microbes like *Botrytis cinerea* (Elmer and Reglinski, 2006; Badawy and
45 Rabea, 2009), *Penicillium expansum* (Liu *et al.*, 2007; Yu *et al.*, 2007), *Alternaria alternata*
46 (Sanchez-Dominguez *et al.*, 2011), *Colletotrichum gloeosporioides* (Ramos-Garcia *et al.*,
47 2012) and *Rhizopus stolonifer* (Bautista-Banos *et al.*, 2006) by inhibiting spore germination,
48 germ tube elongation and mycelial growth of fungal phytopathogens. Application of chitosan
49 on tomato at 1.0, 1.5 and 2.0% concentrations significantly suppressed the development of
50 *Rhizopus stolonifer* as compared with untreated fruit. In yet another study on tomato, it was
51 found that application of chitosan at different concentrations in the range 0.01 to 1%
52 significantly inhibited the growth of ***Botrytis cinerea*** and ***Penicillium expansum*** as compared
53 with untreated tomato. Chitosan exhibits direct fungitoxic activity as well as elicits the
54 biochemical defense responses in fruit (Liu *et al.*, 2007). On the basis of above
55 facts, application of chitosan biopolymer was tested on **tomato** against microbial decay.

56 **Material and method**

57 **Chitosan preparation:** In present study, bulk-chitosan (BCH) formulations were prepared by
58 dissolving chitosan (Mol. Wt. 50,000–190,000 and 80% N-deacetylation; Sigma-Aldrich, St.
59 Louis, MO, USA) into 1% glacial acetic acid to get final concentrations of 0.01, 0.04, 0.08,
60 0.12, 0.16 and 0.2% (w/v) with adjusting pH 5.1 with 1N NaOH. The chitosan formulations
61 thus prepared were used to treat tomato fruits.

62 **Experimental details:** Selected tomato fruits were dipped into different concentrations of
63 bulk chitosan (0.01, 0.04, 0.08, 0.12, 0.16 and 0.20%, w/v in water) along with control
64 (distilled water) for 6 min and stored at ambient temperature. Fungal decay was visually
65 inspected in each treatment considering the extent of fungal **mold** on fruit surface in the scale
66 ranging from 1 to 5 where 1 = normal (no decay on fruit surface), 2 = trace (up to 5% of fruit
67 surface decayed), 3 = slight (5–20% of fruit surface decayed), 4 = moderate (20–50% of fruit
68 surface decayed) and 5 = severe (>50% of fruit surface decayed). Results were expressed as
69 fungal decay index. Overall acceptability of the samples was evaluated through the standard
70 sensory evaluation techniques. The sensory attributes such as taste, flavor and acceptability
71 was rated on a five point hedonic scale (9-Excellent, 7-Very good, 5-Good, 3-Fair, 1-Poor) by
72 selected panel of judges (11 members).

73 **Statistical analysis:** Statistical analysis was performed with JMP software version 12 (SAS,
74 2019) using Turkey Kramer HSD test. Each experiment was repeated twice wherein each
75 treatment consisted of minimum three replicates having five tomatoes each.

76 **Result and discussion**

77 In the present study, solutions of bulk-chitosan at different concentrations (0.01, 0.04, 0.08,
78 0.12, 0.16 and 0.20%, w/v) were prepared in 1% acetic acid. The pH of solutions was
79 adjusted to 5.5 to eliminate the acidic damage to tomato fruits. Microbial decay was visually
80 inspected up to 21 days considering the extent of microbial infection on fruit surface. ~~It was~~
81 ~~recorded on 1 to 5 scales where~~ 1 = normal (no decay on fruit surface), 2 = trace (up to 5% of
82 ~~fruit surface decayed), 3 = slight (5–20% of fruit surface decayed), 4 = moderate (20–50% of~~
83 ~~fruit surface decayed) and 5 = severe (>50% of fruit surface decayed).~~ Decay rate increased
84 with storage time due to microbial infection. The lowest decay (5% at scale of 2) was found
85 in fruits treated with 0.08 and 0.16% bulk-chitosan (Table 1). Microbial decay contributes up
86 to ~70% losses in tomato and is, therefore, very crucial to control it during storage. Bulk-
87 chitosan at 2-4% concentrations considerably controlled gray mould cause by **Botrytis**
88 **cinerea** in wound inoculated tomato fruits (Badawy and Rabea, 2009) whereas, at 0.5-1%
89 concentrations significantly inhibited the growth of gray and blue moulds caused **by Botrytis**
90 **cinerea and Penicillium expansum**, respectively in tomato fruits stored for 21 days at 2°C
91 (Liu *et al.*, 2007). Various concentrations of bulk chitosan considerably controlled decay of
92 strawberry, pomegranate and table grapes during storage (Hajji *et al.*, 2018; Candiret *al.*,
93 2018; Felizianiet *al.*, 2015; Gao *et al.*, 2013). In our results, bulk chitosan at 0.08 and 0.16%
94 concentrations significantly controlled tomato decay up to 21 days of storage (Table 1).

95 Results obtained in present investigation are better as compared with previous findings as
 96 very low concentration of bulk-chitosan comprehensively controlled the decay up to 21 days
 97 of storage at ambient temperature. Positively charged chitosan effectively degrades microbial
 98 cell wall and also boosts plant's immunity by enhancing defense enzymes activities (Bai *et*
 99 *al.*, 1988; Butler *et al.*, 1996). Therefore, chitosan is used to coat fruits and vegetables to
 100 control microbial infection during post-harvest storage. Sensory evaluation is another
 101 important parameter responsible for acceptability of tomatoes by consumers. Gao *et al.*
 102 (2013) studied that flavour of table grape significantly decreased after 15 days of storage in
 103 untreated fruits while with chitosan treatment, the sensory parameters were maintained up to
 104 the end of storage period. Our results showed that 0.16% bulk-chitosan was fairly effective to
 105 preserve color, texture, flavour and overall acceptability of tomato fruit (Table 2).

106 **Table 1: Decay at ambient temperature of tomato treated with different**
 107 **concentrations of BCH**
 108

Treatment (%)	Decay			
	Day 0	Day 7	Day 14	Day 21
Control (Water)	0% (No decay)	20%(at scale of 3)	50%(at scale of 4)	>50% (at scale of 5)
Bulk-chitosan				
0.01	0% (No decay)	5%(at scale of 2)	20%(at scale of 3)	50% (at scale of 4)
0.04	0% (No decay)	5%(at scale of 2)	20%(at scale of 3)	>50% (at scale of 5)
0.08	0% (No decay)	0%(No decay)	0%(No decay)	5% (at scale of 2)
0.12	0% (No decay)	0%(No decay)	5%(at scale of 2)	20% (at scale of 3)
0.16	0% (No decay)	0%(No decay)	0%(No decay)	5% (at scale of 2)
0.20	0% (No decay)	5%(at scale of 2)	50%(at scale of 4)	>50% (at scale of 4)

109 **Table 2: Sensory evaluation at ambient temperature of tomato treated with different**
 110 **concentrations of BCH**

Treatment (%)	Sensory Evaluation			
	Day 0	Day 7	Day 14	Day 21
Control (Water)	9.0	7.0	3.0	1.0
Bulk-chitosan				

0.01	9.0	7.0	5.0	3.0
0.04	9.0	7.0	5.0	3.0
0.08	9.0	9.0	7.0	7.0
0.12	9.0	7.0	5.0	3.0
0.16	9.0	9.0	9.0	7.0
0.20	5.0	7.0	5.0	1.0

111 **Conclusion**

112 The available review of literature and results of the present investigation concluded that
 113 amongst the various treatments, 0.16% bulk-chitosan significant results in microbial decay
 114 and sensory evaluation as compared with control up to 21 days of storage at ambient
 115 temperature ($27\pm 2^{\circ}\text{C}$). Based on the aforementioned results and comparative evaluation of
 116 different doses of bulk-chitosan, we concluded that chitosan are very effective at less
 117 concentration and thus exert minimum chemical load on the treated tomatoes. Therefore, it is
 118 possible to assert that chitosan biopolymer has the capacity to shield tomatoes against
 119 microbial degradation.

120 **Reference**

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