

1 **Effect of chitosan biopolymer on microbial decay in tomato (*Solanum*** 2 ***lycopersicum* L.) variety 'Dev' under ambient temperature**

3 4 **Abstract**

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

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

16 17 **Introduction**

18 "Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop after potato
19 (FAO, 2021), and it is a model plant for researchers, especially for those studying the growth,
20 yield and quality of fleshy fruits" (Bertin and Genard, 2018). "Tomato is one of the most
21 important foods for humanity. The post-harvest maintenance of food is configured as one of
22 the objectives to be achieved in the sustainable development proposed by FAO in 2030.
23 Tomato contains higher amounts of lycopene, a type of carotenoid with antioxidant properties
24 which is beneficial in reducing the incidence of chronic diseases like cancer and many other
25 cardiovascular disorders" (Viskeliš *et al.*, 2015). India is the 2nd largest (11.5%) producer of
26 tomato in world. According to FAOSTAT (2021), "tomato was grown world-wide in an area
27 of 5.16 MH with the production of 189.13 MT and productivity of 36.6 MT per hectare. In
28 India it was grown in 0.84 MH with the production of 21.18 MT and productivity of 25.06
29 MT per hectare". "Chitosan is a linear amino polysaccharide of glucosamine andN-
30 acetylglucosamine units and obtained by alkaline deacetylation of chitin extracted from the

31 exoskeleton of crustaceans such as shrimps and crabs as well from the cell walls of some
32 fungi” (Sucharitha *et al.*, 2018). “It is being widely used in different fields ranging from
33 medicine, tissue and bone engineering to food sector, cosmetics, textiles, pharmaceutical,
34 biotechnology, paper industry and in waste-water treatment” (Jiang and Li, 2005). In
35 agriculture, chitosan biopolymer is an ideal antimicrobial agent in preservation of fruits and
36 vegetables. Chitosan is generally used to prevent microbial diseases and decay of fruits and
37 vegetables through its pre- and post-harvest treatments (Bautista-Banos *et al.*, 2006).
38 Chitosan is considered as a strong antimicrobial agent due to its positive surface charge
39 which alters the microbial membrane structure that eventually leads to leakage of cellular
40 fluids and death (Goyet *et al.*, 2009). The NH₂ group present in it is responsible for its
41 bioactivity against microbial community through inhibition of gene expression and protein
42 synthesis (Kong *et al.*, 2010). Chitosan has been used to prevent post-harvest losses in fruits
43 and vegetables. It is also effective in reducing post-harvest diseases caused by various
44 microbes like *Botrytis cinerea* (Elmer and Reglinski, 2006; Badawy and Rabea, 2009),
45 *Penicillium expansum* (Liu *et al.*, 2007; Yu *et al.*, 2007), *Alternaria alternata* (Sanchez-
46 Dominguez *et al.*, 2011), *Colletotrichum gloeosporioides* (Ramos-Garcia *et al.*, 2012) and
47 *Rhizopus stolonifer* (Bautista-Banos *et al.*, 2006) by inhibiting spore germination, germ tube
48 elongation and mycelial growth of fungal phytopathogens. In yet another study on tomato, it
49 was found that application of chitosan at different concentrations in the range 0.01 to 1%
50 significantly inhibited the growth of *Botrytis cinerea* and *Penicillium expansum* as compared
51 with untreated tomato. Chitosan exhibits direct fungitoxic activity as well as elicits the
52 biochemical defense responses in fruit (Liu *et al.*, 2007). On the basis of above facts, in
53 present study, we proposed chitosan formulation, and delve its bioactivity by testing its effect
54 on tomato variety ‘Dev’. Efforts have been made to study the chitosan formulation against
55 microbial decay and sensory evaluation under ambient temperature upto 21 days.

56

57 **Material and method**

58 **Chitosan preparation:** In present study, bulk-chitosan (BCH) formulations were prepared
59 by dissolving chitosan (Mol. Wt. 50,000–190,000 and 80% N-deacetylation; Sigma-Aldrich,
60 St. Louis, MO, USA) into 1% glacial acetic acid to get final concentrations of 0.01, 0.04,
61 0.08, 0.12, 0.16 and 0.20% (w/v) with adjusting pH 5.1 with 1N NaOH. The chitosan
62 formulations thus prepared were used to treat tomato fruits.

63 **Experimental details:** The experiment was conducted during (September to November)
64 2018-2019 at the Department of Horticulture and Department of Molecular Biology and
65 Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture
66 and Technology, Udaipur. Tomato variety *Dev* grown at the University farm, Rajasthan
67 College of Agriculture, MPUAT was used in present investigation. Tomatoes with similar
68 size, firmness and colour were selected for the experiment. The fruits with visible decay and
69 mechanical damage were abandoned for the study. Selected tomato fruits were dipped into
70 different concentrations of chitosan formulation (0.01, 0.04, 0.08, 0.12, 0.16 and 0.20%, w/v
71 in water) along with control (distilled water) for 6 min and stored at ambient temperature.

72 **Fungal decay index:** Mechanical cut (0.5 cm x 1.0 cm) was positioned on tomatoes for
73 stimulation of microbial infection. "Microbial decay was visually examined, considering the
74 level of fungal growth on the tomato surface in the scale ranging from 1 to 5, where 1 =
75 normal (no decay on fruit surface), 2 = trace (up to 5 % of fruit surface decayed), 3 = slight
76 (5–20 % of fruit surface decayed), 4 = moderate (20–50 % of fruit surface decayed) and 5 =
77 severe (>50 % of fruit surface decayed)" (Babalar *et al.*, 2007). Results were expressed as
78 fungal decay index.

79 **Overall organoleptic score**

80 Overall acceptability of the samples was evaluated through the standard sensory
81 evaluation techniques. The sensory attributes such as taste, flavor and acceptability was rated
82 by using five-point hedonic scale (9-Excellent, 7-Very good, 5-Good, 3-Fair, 1-Poor) as well
83 as 11 members panel of judges (Post harvest experts).

84 **Statistical analysis:** Statistical analysis was performed with JMP software version 12 (SAS,
85 2019) using Turkey Kramer HSD test. Each experiment was repeated twice wherein each
86 treatment consisted of minimum three replicates having five tomatoes each.

87 **Result and discussion**

88 In the present study, solutions of bulk-chitosan at different concentrations (0.01, 0.04, 0.08,
89 0.12, 0.16 and 0.20%, w/v) were prepared in 1% acetic acid. The pH of solutions was
90 adjusted to 5.5 to eliminate the acidic damage to tomato fruits. Microbial decay was visually
91 inspected up to 21 days considering the extent of microbial infection on fruit surface. Decay
92 rate increased with storage time due to microbial infection. The lowest decay (5% at scale of
93 2) was found in fruits treated with 0.08 and 0.16% bulk-chitosan. Microbial decay contributes

94 up to ~70% losses in tomato and is, therefore, very crucial to control it during storage (Meena
95 *et al.*, 2020). Bulk-chitosan at 2-4% concentrations considerably controlled gray mould in
96 wound inoculated tomato fruits (Badawy and Rabea, 2009) whereas, at 0.5-1%
97 concentrations significantly inhibited the growth of gray and blue moulds in tomato fruits
98 stored for 21 days in cold storage (Liu *et al.*, 2007). Various concentrations of bulk chitosan
99 considerably controlled decay of strawberry, pomegranate and table grapes during storage
100 (Hajji *et al.*, 2018; Candir *et al.*, 2018; Feliziani *et al.*, 2015; Gao *et al.*, 2013). In our results,
101 bulk chitosan at 0.08 and 0.16% concentrations significantly controlled tomato decay up to
102 21 days of storage (Fig. 1). Results obtained in present investigation are better as compared
103 with previous findings as very low concentration of bulk-chitosan comprehensively
104 controlled the decay up to 21 days of storage at ambient temperature (El Ghaouth *et al.*,
105 1992). Positively charged chitosan effectively degrades microbial cell wall and also boosts
106 plant's immunity by enhancing defense enzymes activities (Bai *et al.*, 1988; Butler *et al.*,
107 1996). Sensory evaluation is another important parameter responsible for acceptability of
108 tomatoes by consumers. Gao *et al.* (2013) studied that flavour of table grape significantly
109 decreased after 15 days of storage in untreated fruits while with chitosan treatment, the
110 sensory parameters were maintained up to the end of storage period. Our results showed that
111 0.16% bulk-chitosan was fairly effective to preserve color, texture, flavour and overall
112 acceptability of tomato fruit (Fig. 2).

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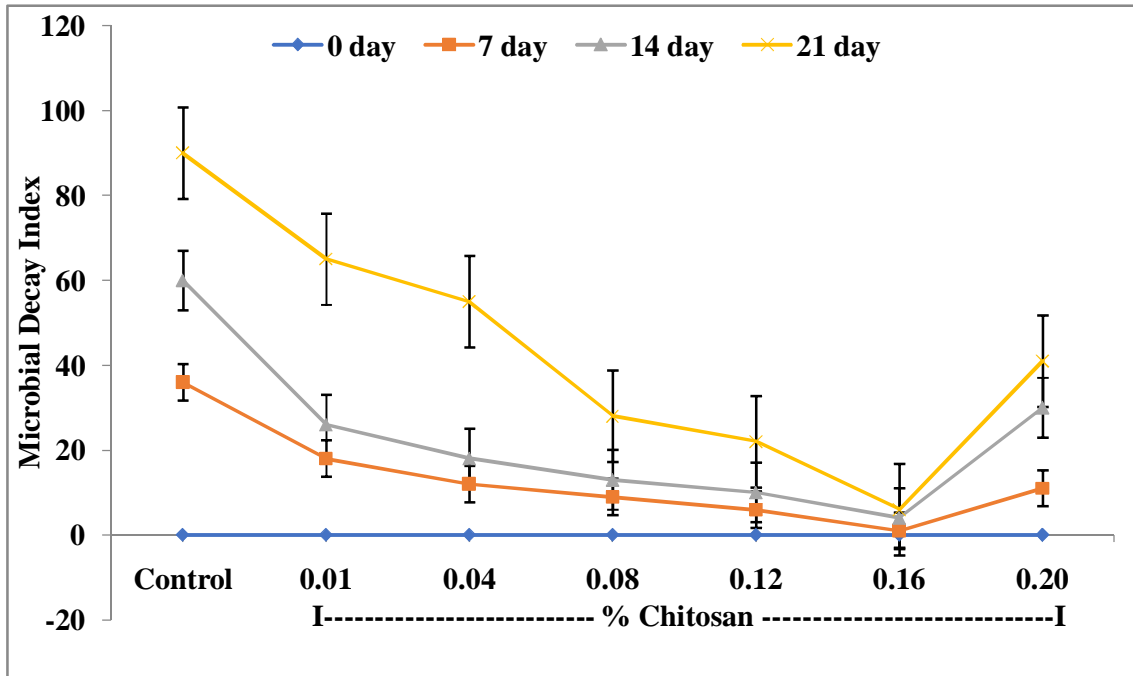
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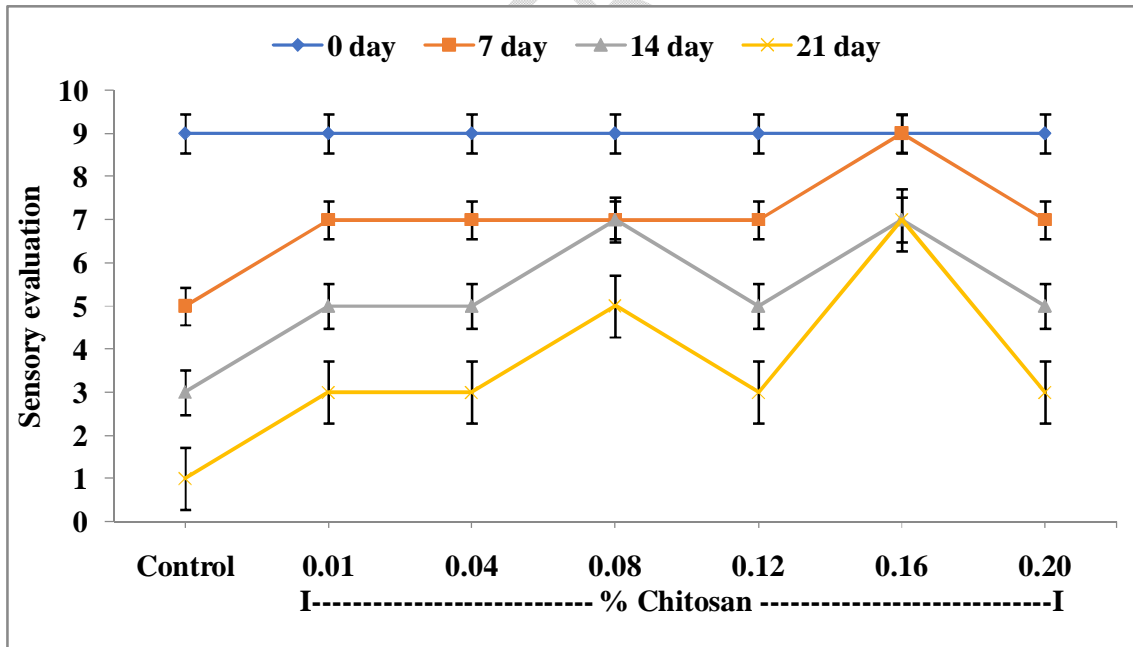
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120 **Fig. 1:** Effect of chitosan formulation on microbial decay at room temperature of tomato.
 121 Each value is mean of triplicate. Error bars represents \pm SE.



122

123 **Fig. 2:** Effect of of chitosan formulation on sensory evaluation at room temperature of
 124 tomato. Each value is mean of triplicate. Error bars represents \pm SE.

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126

127 **Conclusion**

128 The available review of literature and results of the present investigation concluded that
129 amongst the various treatments, 0.16% bulk-chitosan significant results in microbial decay
130 and sensory evaluation as compared with control up to 21 days of storage at ambient
131 temperature. Based on the aforementioned results and comparative evaluation of different
132 doses of bulk-chitosan, we concluded that chitosan are very effective at less concentration
133 and thus exert minimum chemical load on the treated tomatoes. Therefore, it is possible to
134 assert that chitosan biopolymer has the capacity to shield tomatoes against microbial
135 degradation.

136 **Reference**

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