USE OF BIO-NANOCOMPOSITES IN ENHANCING BACTERIAL WILT PLANT RESISTANCE, TOMATO PRODUCTION AND WATER CONSERVATION IN GREENHOUSE FARMING

D. M. Gatahi[1], H. Wanyika[2], A. W. Kihurani[3], E. Ateka[4] and A. Kavoo[5]
[1,4]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology
[2]Department of Chemistry; Jomo Kenyatta University of Agriculture and Technology, Nairobi
[3,5]Department of Crop Science; Karatina University, Karatina
Email: denmagkenya@gmail.com

Abstract
This study was conducted to examine the effects of bio-nanocomposites in increasing bacterial wilt resistance, growth and development and water conservation in tomato production in a greenhouse. The experiment was conducted using completely randomized design (CRD) with 19 treatments and 3 replications. The effect on resistance elicitation in different tomato varieties was analysed using polymerase chain reactions (PCR) by determining the presence and concentration of chitinase and glucanase. Enhanced resistance was determined using bioassays on reported wilt incidences in tomato seeds and seedlings treated with bio-nanocomposite and challenged with the *Ralstonia solanacearum*. Effect of bio-nanocomposites in tomato plants yield and shelf life was determined in form of the obtained yield and longevity of stored tomato fruits after harvesting. Water conservation in propagated seedlings and tomato plants rhizosphere growing in the greenhouse was determined using a digital potential meter based on percent moisture loss after irrigation. Enhancement of the two genes (chitinases and glucanases) in the DNA tests coupled with reduced wilting of the tomato crops in bio-nanocomposites treatments indicated induced resistance. In contrast, tomato seedlings grown in the control, had reduced growth and were significantly ($P<0.05$) infected by the wilting disease. There was significant difference in yield and shelf life between the treatments and controls ($P<0.05$). In addition, the nanocomposite significantly resulted in enhanced water conservation ($P<0.05$). The BCAs and chitosan silica nanocomposites are able to increase physiological, biochemical and soil microbial activities enhancing tomato production and resource utilization efficiency. The study therefore, heralds the era of using bionanocomposites in induction of host plant resistance, improved tomato productivity, increased tomato fruit shelf life and water conservation due to the resultant synergies of the constituent elements.

Key words: Chitosan immobilized silica nanocomposites (CISNC); Biological antagonists; chitinases; glucanases; yield; post harvest losses.

1.0 Introduction

Optimal tomato production in the greenhouses is affected by a myriad of factors. Such factors include; soil fertility, pest and diseases and water economics. These factors affect the yield obtained thus affecting profitability (KHCP, 2012). Among the most reliable pests and disease control mechanisms is the host plant resistance. The method entails use of plants with an innate resistance to pests by either resisting or overcoming infestation. Elicitation of resistance is an emerging technology where plants with low host plant resistance are treated with resistance inducing agents to increase resistance to pests and disease pathogens (Chaumpluk *et al*., 2012).

The study entailed treatment of different tomato varieties with resistance elicitors like the chitosan, silica and microbial antagonists to increase resistance in the host-plant defense system. Effect of bionanocomposites applied on soil water retention was also determined. This was done as tomato production in a greenhouse requires copious amounts of water due to increased evapotranspiration. Thus any measure that enhances water utilisation efficiency has potential of increasing productivity and sustainability of greenhouse farming. Harvested produce was treated with the bio-nanocomposites to determine effect on shelf life. This is because, about 30% of harvested tomato fruit yield is lost through post harvest losses in Kenya. The loss is attributed to physiological and pathological effects. (KHDP, 2007). Data on yield per treatment was taken to determine effect of different BCA-nanocomposite treatment on yield.

The use of BCAs-nanocomposites in enhancing host plant resistance and post harvest shelf life has been attributed to the antimicrobial effect of the useful microbes, chitosan and silica materials. The materials posses' antimicrobial effect individually (Agrios, 2005). Previous work in this study, showed compatibility when the microbes were incorporated to chitosan-silica nanocomposites and synergy in efficacy when applied together (Gatahi *et al*., 2015). Application of BCAs and nanocomposite carriers increases the microbial activity in the rhizosphere. Use of organic carriers also increases the longevity of microbes in the soil and their efficacy

in root hairs colonisation. The BCAs-nanocomposites also increase plant productivity due to their role in plant nutrition, water utilization efficiency and pathogen inhibition (Sood, 2003).

2.0 Experimental

2.1 Materials

All materials were analytical grade and included; MSN, acetic acid, NaOH pellets, Tri-poly phosphate (TPP), chlorox, tetrazolium chloride (TZC) were obtained from Sigma Aldrich. BCAs (*Glomus mossease*, effective micro-organisms, *Trichoderma viridae* and *Bacillus subtilis*) were obtained from Juanco Co. Ltd and Real IPM for the 1st two and last two respectively. *R. solanacearum* phage was isolated from infected greenhouse soil. Chitin was obtained from Laborex while nutrient agar, potato dextrose agar, master mix PCR kit and primers were obtained from Bioneer Ltd.

2.2 Synthesis of Chitosan Immobilized Silica Nanocomposites

A 500 mg sample of MSN was dispersed in 100 ml phosphate buffered saline (PBS) to form a partial solution. Solubilised chitosan nanoparticles (50 ml) were added to 100 ml of MSN suspension. The mixture was vortexed for 2 mins and placed in a vibratory shaker for 2 hrs then magnetic stirrer for 2 hr. The excess suspension of MSN that was not adsorbed in the chitosan gel matrix was poured carefully and disposed leaving behind a gelly substance of chitosan-silica nanocomposites. A drop of 25% glutaraldehyde was added to the chitosan nanoparticles-MSN mixture using a syringe. The mixture was vortexed and placed on a magnetic stirrer for 1 hr (Kubata *et al*., 2005) and (Zhang *et al*., 2002). This resulted in the formation of chitosan immobilised silica nanocomposites gel denoted CISNC.

2.3 Preparation of Biocontrol Agents' Nanocomposites

Chitosan immobilized silica nanocomposites were prepared following the procedure of Gatahi *et al*, 2015. The nanocomposite was used for adsorbing biocontrol agents including: *Bacillus subtilis*, *Glomus mossease*, *Trichoderma viridae*, *R. solanacearum* phage and effective micro-organisms (EM). The microbes were cultured on the respective growth media that is, nutrient and potato dextrose agar for bacteria and fungi respectively. A cellular suspension was prepared and standardized to 2.000 O.D using Shimadzu Ultra violet visible (Uv-vis) spectrophotometer and adsorbed on 10% chitosan immobilized silica nanocomposites (CISNC) and chitosan immobilized nanoclay (CINC). The nanocomposites and bio-nanocomposites were characterized on Rigaku X-ray powder diffractometer. A suspension of 10% was prepared (1:10 bio-nanocomposite to distilled water) was prepared for inoculation.

2.4 Experimental Design

The microbial and bio-nanocomposite complex were then applied on tomato seeds prior to seeding by priming. A similar treatment was done on the planting media (cocopeat) and the primed seeds sown on a matching treatment in a tray. The seedlings were also treated with a similar complex prior to transplanting. Effect of different treatments on resistance elicitation in tomato varieties was tested inside a greenhouse using a completely randomized design with 19 treatments, 3 replications and 2 controls.

2.5 Biochemical Analysis (Glucanases and Chitinases)

The efficacy of resistance elicitation was carried out by determining the levels of chitinases and glucanases biochemicals. A confirmatory test of the presence of the chitinases and glucananses enzymes was done after amplification of DNA by use of polymerase chain reaction (PCR).

Foliage from treated tomato plants was ground to obtain a suspension for DNA isolation. DNA was extracted following the CTAB extraction method and then stored at -20^{0}C (Kumlachew, 2014). The polymerase chain reaction (PCR) was carried out using touchdown procedures as described by Khalil *et al*. (2003). The primers were a 21 mer forward primer –CGA ACC TAA TGG TGG TAG TGC-, and reverse –TCG CAA CTA AAT CAG GGT TG- for chitinases. A 22 mer forward primer –CGC CAT TGC TCG TGT TGA CAT G- and reverse -AAT TTC TCG CTC GGC GGT GGT G for glucanases. The samples were cooled at 4^{o}C and subjected to electrophoresis on a 1.5% agarose gel in 1X TAE buffer (40 mM Tris acetate and 1.0 mM EDTA) and photographs taken under ultra-violet (Uv) light. The obtained ladders were interpreted using base pair amplicons to the enzymes (Chilvers, 2012**)**.

Expression of resistance associated genes in the DNA that is chitinase and glucanase were determined using PCR analysis of tomato plant foliage.

Amplified DNA (100 µL) was mixed with a binding buffer in a ratio of 1:1 mixed thoroughly by vortexing and 10 µL of 3 M sodium acetate added and vortexed until a yellow colour appear. A solution (800 µL) was transferred

to the GeneJETpurification column, Centrifuged for 30-60 s and the flow-through discarded. Wash buffer (700 µL) was added to the GeneJET purification column, centrifuged for 30-60 s, flow-through discarded and the purification column placed back into the collection tube. The empty GeneJET purification column was centrifuged for 1 min. The GeneJET purification column was transfered to a clean 1.5 mL microcentrifuge tube and centrifuged for another 1 min. the GeneJET purification column was discarded and the purified enzyme stored at -20°C. Enzyme concentration was determined using Bioneer, nanodrop machine at 680/620 nm on purified chitinases and glucanases enzymes prepared.

2.6 Disease Incidence

To assess bio-nanocomposite complex efficacy on induced wilt resistance, incidences of bacterial wilt disease symptoms were observed and recorded as number of wilting plants per treatment. Wilting incidence was calculated using the formula;

$$\frac{(5A+4\ B+3\mathrm{C}+2\mathrm{D}+\mathrm{E})}{1.75\ N} \quad \text{.....i}$$

Where, A=number of plants on scale 5; B=number of plants on scale 4; C=number of plants on scale 3; D=number of plants on scale 2; E=number of plants on scale 1; N=total number of plants. From the scale, the lower incidence level the better the control measure (Tim *et al*., 2008).

2.7 Water Moisture Loss Determination

Water moisture was measured using a digital moisture meter. Measurements were done before and after irrigation. The difference in soil moisture was taken to show moisture loss. The water loss was expressed as a percentage of initial and the retained moisture contents.

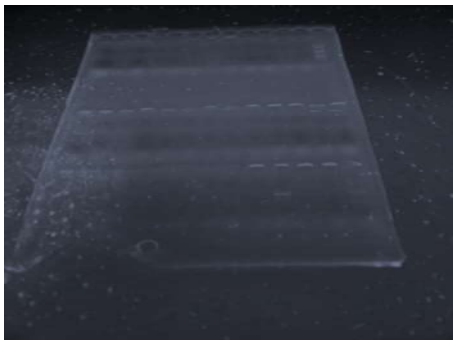
3.0 Results and Discussions

3.1 Expression of Resistance Genes and Enzymes

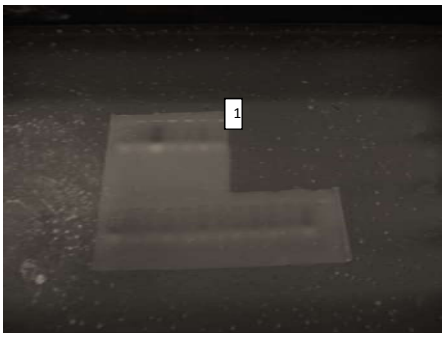
Effect of treating tomato plants with BCA-nanocomposite complexes were expressed by induced pathogenesis related enzymes in the tomato plant system in form of chitinases and glucanases.

BCAs, chitosan-silica nanocomposites and their complexes increased concentration of chitinase and glucanase significantly ($P<0.05$) when compared to the controls. The expression of the enzymes was also confirmed by amplification of the DNA. Data on concentration of the two enzymes and their expression is shown in table 4 and plate 1 respectively.

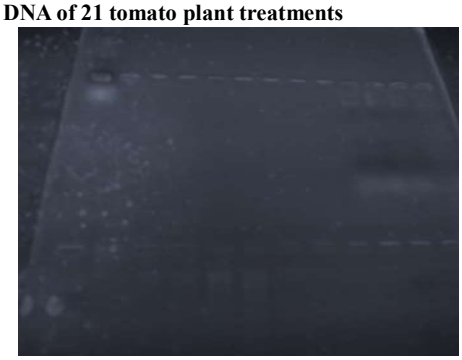
The images for chitinases and glucanases DNA are described in Plate 1 while the enzymes concentrations are corroborated in table 1 and 2.



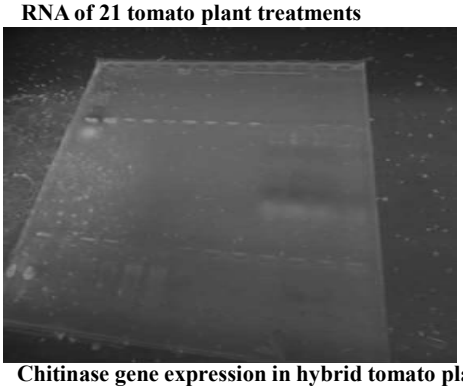
DNA of 21 tomato plant treatments



RNA of 21 tomato plant treatments



Glucanase gene expression in hybrid tomato plants



Chitinase gene expression in hybrid tomato plants

Plate1. Isolated and amplified DNA of chitinase and glucanase enzymes
Source: Gatahi et al., 2015.

Table 1: Concentration of chitinase enzyme in tomato plants treated with bionanocomposites

Treatment	Chitinases	
11	1.06a	a
5	1.167a	b
4	1.233c	c
12	1.267cd	cd
15	1.317de	de
2	1.337e	e
13	1.417f	f
14	1.427f	f
16	1.427f	f
1	1.563g	g
8	1.607g	g
3	1.697h	h
19	1.707h	h
7	1.76i	i
17	1.93j	j
10	2k	k
18	2.037k	k
9	2.24l	l
6	2.61m	m

Means linked with a similar letter are not significantly different. LSD $_{0.05}$

Table 2.Concentration of glucanase enzyme in tomato plants treated with bionanocomposites

Treatment	Glucanases	
5	0.1233a	a
4	0.1333ab	ab
11	0.1467ab	ab
2	0.15ab	ab
13	0.1733b	b
15	0.2333c	c
1	0.2433c	c
14	0.2567cd	cd
12	0.2667cd	cd
3	0.2667de	de
19	0.2667de	de
16	0.2667ef	ef
7	0.2667ef	ef
8	0.2667f	f
17	0.2667g	g
10	0.2667h	h
6	0.2667hi	hi
18	0.2667i	i
9	0.2667j	J

s.e 0.02117
cv (%) 6.7
Means linked with a similar letter are not significantly different. LSD $_{0.05}$

Plant materials were taken after the 8th week as optimal biochemicals and growth occurs at this stage. The enhanced resistance was expressed by elevation of chitinases and glucanases. The two hydrolysis enzymes were chosen as resistance markers due to their association with response to pests. The study showed high correlation (R^2- 0.917*, P<0.05) between the induced chitinase and glucanase concentration.
Garcia-Garrido and Ocampo, 2002 demonstrated that some mRNA of genes associated with plant defense response accumulated in plants with mycorrhizal structures such as the hyphae, vesicules and arbuscules. Colonized plants also had elevated defense related genes and metabolism enzymes. Chitinase and glucanase are synergistically induced during attack by pathogens and/or resistance elicitors. Though Pozo *et al*., 2002 opined that the enzymes were non-specific defense response initiated in the plants, there was evident and systematic reduction of disease symptoms in tomato plants with the elevated enzymes. This resulted in the enzymes being referred to as bioprotector agents.
According to Garcia-Garrido and Ocampo, 2002, total chitinases activity is higher in mycorrhizal friendly plants more so when colonized by the root-fungus complex than non-mycorrhizal plants and their controls. In proving association between the chitinase and glucanase activity and BCAs, Garcia-Garrido and Ocampo, 2002, found out that the two enzymes were low before tomato plants were colonized with mycorrhiza. Raczyska-Bojanowska, K (1998) found out that constitutive activities of chitinase and glucanase were several fold lower in wheat leaves before treatment with elicitors. The enzymes were elevated significantly (P<0.05) upon treatment with *Stagonospora nodorum* isolates with high virulence.

Participation of the hydrolytic enzymes; chitinase and glucanase in defense response of plants has also been described by Hahlbrock *et al*., 1987. This has been attributed to the fact that most pathogenic bacteria and fungi contain 1, 3 B-glucans, chitin and other substrates of these enzymes as cell wall components. These enzymes, therefore effectively restrict growth of fungi and bacteria as they have lysozyme activity. Infection of healthy plants by pathogens is also associated with rapid activation of the corresponding gene that is, chitinase and/or glucanase gene(s) around the necrotic area in parsley leaf.

3.2 Wilt Incidences in Tomato Plants after Treatment with Bionanocomposites

Tomato seedlings treated with BCA-CISNC complex particularly the effective micro-organisms and phages had the least wilt incidences. Wilt incidences in tomato plants treated with BCA-nanocomposite is shown in Table 3.

Table 3: Wilt incidences in tomato seedlings treated with bio-nanocomposites

Treatment	**Mean**	
6 CISNC-EM	19.62	a
5 CISNC-BS	20.50	a
9CISNC-PHAGE	25.38	ab
8 CISNC-AMF	26.25	ab
4CISNC	27.62	abc
19CINC	28.12	abc
7CISNC-TV	29.00	abc
12 EM	29.75	abcd
17MSN	33.12	bcd
3CHTNP	35.88	bcde
14AMF	36.50	bcde
2CHT	36.62	bcde
10PHAGE	38.75	cde
13TV	39.50	cde
11 BS	41.75	de
1CHITIN	46.00	ef
16AA	54.50	fg
18DW	55.88	fg
15RS	62.88	g

Means linked with a similar letter are not significantly different. LSD $_{0.05}$

Wilt incidences least occurred in BCAs-CISNC and CISNC only and CISNC-nanoclay composite treatments compared to plants treated with BCAs only or other nano-composites only. Control plants (acetic acid and distilled water) had significantly ($p \leq 0.05$) higher wilt incidences. Increased resistance by BCAs is attributed to, production of antibiotics, hormones and cyanic acid and by competition of colonization sites, carbon components and induction of systemically induced resistance for disease suppression.

Use of chitosan in the nanocomposite carrier enhanced the BCAs' efficacy against the pathogen. When chitosan nanocomposite was combined with microbial antagonists, like the *B. subtilis*, effective micro-organisms, T. *viridae, G. mossease* and *R. solanacearum*-phage, their efficacies were enhanced. This shows that chitosan is a good carrier material, a characteristic attributed to the polysaccharides nature of the compound. According to El-Hadrami *et al*., 2010, chitosan stimulated microbial degradation of pathogens in a way resembling the application of a hyper-parasite. Part of the effect observed by chitosan on reduction of soil borne pathogens comes from the fact that it enhances plant defense responses. The other part is linked to the fact that this biopolymer is composed of polysaccharides that stimulate the activity of beneficial micro-organisms such as *Bacillus spp*. and *Mycorrhiza spp*. altering the microbial equilibrium in the rhizosphere disadvantaging plant pathogens.
Chitosan and its derivatives are also degraded producing pathogen repellents like ammonia which pre-dispose the *R. solanacearum* pathogen to the emboldened biological antagonists making the adsorbed miro-organisms more efficacious in controlling the pathogen as observed in this study. However, according to Pal and Spadden, 2006, BCAs are more likely to be preventive than therapeutic on disease thus their potential should be harnessed by seed priming and/or pre-treatment before transplanting. The BCAs were found to be more efficacious in seed primed seedlings while chitosan and its derivatives acted better as a soil drench (Algam *et al*., 2010).

The reduction of wilt caused by *R. solanacearum* pathogen in nanocomposite containing silica, was attributed to the fact that Silicon augments resistance in tomato seedlings. This is because, though tomato is not a silicon accumulating plant, soluble Silicon is absorbed and accumulated in the apoplast particularly the epidermal cell walls. Assimilated Silica in plants inhibits fungal and bacterial diseases by physically inhibiting penetration of the epidermis through lignification of the membranes (Balakhina and Borkowska, 2013. Silicon is a precursor in the synthesis of lignin. Hence, improves seed coat resistance, decreases seed susceptibility to mechanical damage and metabolite leaching. In contrast to the above observation, Datnoff *et al.*, 1997 found out that Silicon did not significantly ($P<0.05$) improve a susceptible cultivar resistance. However, Jian, 2004, proved that application of Silicon led to activation of Pathogenesis-related proteins such as Catalase, peroxidase, polyphenol oxidase, glucanase, chitinase in a pathogen infected plant. The proteins are associated with in increased plant resistance to pathogens. This was in agreement with the current study where tomato seeds and seedlings treated with MSN and its derivatives were more resistant to *R. solanacearum* pathogen.
Formation of a chitosan-MSN composite increased the role of chitosan manifold as there was significant difference ($P<0.05$) of wilt incidences in tomato seedlings treated with MSN and chitosan-MSN nanocomposite. The nanocomposite also had better sorption properties than MSN. This was attributed to the increased active sites for reaction due to the gel forming properties of the composite. According to Mandal *et al.,* 2013, induced lignification and antimicrobial biochemicals, could have played an important role in host plant resistance of tomato plants in this study.

3.3 Yield of Tomato Plants After Application of Bionanocomposites

Harvested tomato fruits from plants treated with BCAs, Chitosan-silica nanocomposites and their complexes had a significant ($P< 0.05$) effect on yield of tomato fruits compared to the controls. Phage treatment did not result in a significant effect on yield. Data on effect of the treatments on the yield is shown in table 4 and 5 for the fruit sizes (cm) and obtained weight (kg) respectively. The tomato growth is also corroborated on plate 2.

Table 4: Size (Θ cm) of tomato fruits after harvested after treatment with bio-nanocomposites

Treatment	Means
11DW	15.00 a
12AA	15.50 ab
10PHAGE	15.75 b
9TV	16.00 b716.62 c
16CHT	17.12 cd

2	17.38 de
3CISNC-PHAGE	17.38 de
6CISNC	17.38 de
13MSN	17.75 ef
17NANOCLAY	17.83 ef
5CISNC-BS	18.12 f
14EM	18.12 f
4CISNC-EM	18.88 g
15AMF	19.00 g
8CHTNP	19.88 h
1CISNC-AMF	20.00 h

Means linked with a similar letter are not significantly different. LSD $_{0.05}$

Table 5 Average weight of tomato fruits (Kgs/plant)

Treatment	**Means**
11DW	5.718 a
12AA	6.270 ab
10PHAGE	7.400 bc
9TV	7.750 cd
13MSN	8.203 cde
17NANOCLAY	8.018 cde
7BS	8.535 cdef
14EM	8.870 defg
16CHT	8.920 defg
3CISNC-PHAGE	8.948 defgh
8CHTNP	9.428 efghi
15AMF	9.675 fghi
CISNC-TV	9.728 fghi
5CISNC-BS	9.988 ghi
6CISNC	10.288 hi
18	10.342 hi
18	10.341 hi
4CISNC-EM	10.508i
1 CISNC-AMF	12.713 j

Means linked with a similar letter are not significantly different. LSD $_{0.05}$

Plate 2: Image of tomato plants treated with bio-nanocomposites
Source: Gatahi et al., 2015

Key: Treatments
1 chitosan immobilized silica adsorbed with Glomus mosseae (Mycorrhiza) CISNC-AMF, 2 CISNC-TV(Trichoderma viridae), 3CISNC-phage, 4CISNC- EM (Effective micro0organisms), 5CISNC-BS (Bacillus subtilis), 6CISNC, 7bs, 8 Chitosan nanoparticles (Chtnp), 9TV, 10phage, 11distilled water (DW), 12acetic acid (AA), 13 Mesoporous silica nanoparticles (msn), 14 EM, 15AMF, 16 Chitosan (cht), 17 nanoclay, 18 chitosan-nanoclay (CINC).

The crop yield can by greatly improved by optimum doses of Si application, which is due to increasing chlorophyll contents. Effects of silicon deposited in leaves on improving photosynthetic potential and efficiency by opening angle of leaves, keeping the leaf erect, and decreasing self-shading have been reported in rice (*Oryza sativa* L.), barely (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and sugarcane (*Saccharum officinarum* L.) (Soratto *et al*., 2012). Photosynthetic capacities of crops' applied Si are improved by the enlarged size of chloroplasts and the increased number of grana in leaves (Yang, 2010).

The enhanced growth rate was attributed to the role of BCAs as biostimulants and provision of nutrients in the rhizosphere. Chitosan played a role in stimulating growth of beneficial microbes due to high carbon content in the polymer. The activated microbes accelerated decomposition of organic matter into inorganic forms. The BCAs in addition, enhanced root system development enabling the plants to absorb more nutrients from the soil. Chitosan also caused chelation of nutrients and acted as a fertilizer due to the high Nitrogen and Carbon contents. Cao *et al*., 2013 found out that, chitosan contains oligosaccharides that act on plants as phytohormones. Hence, regulating the processes of morphogenesis, growth and development. It also promotes plant growth through increasing availability and uptake of water and essential nutrients by adjusting osmotic pressure. Chitosan treatment as observed in Table 3, increased chlorophyll content in tomato plants, marking increased intensity of net photosynthesis (Li *et al*., 2010. It also reduces transpiration rate without major effect on plant biomass by altering stomatal opening and closure mechanisms. Chitosan is therefore considered as a water utilization efficient agent (Dzung *et al*., 2013. Guan *et al.,* 2009, found out that, chitosan enhanced germination index, reduced mean germination time, increased shoot height, root length, shoot and dry weights. It also promoted the growth of microbial species with antagonistic action against pathogens.
Increased plant growth is also associated with enhanced inorganic nutrient absorption, that results in higher photosynthesis thus photosynthates accumulation (Gau *et al*., 2000). Chitosan increases water and mineral uptake in plants. Is also act as a fertilizer due to the rich nitrogen and carbon contents. Inoculation of plants with BCAs, significantly improved growth parameters. For instance, AMF, have been found to increase plant growth (Mortimer et al., 2008), increase chlorophyll content (Demir, 2004), phosphorous content (Gaur *et al*., 2000, increase resistance to cultural and environmental stresses and consequently improve plant growth (Smith and Read, 1997).

3.4 Effect of Treating Tomato Fruits with Bionanocomposites on Shelf Life
Treatment of tomato fruits with Chitosan-silica nanocomposites and BCA-nanocomposites increased the shelf life of tomato fruits significantly ($P<0.05$) compared to the controls. Adsorption of BCAs on chitosan-silica nanocomposites enhnanced their efficacy in increasing tomato fruits shelf life. The results of bionanocomposites treatment of tomato fruits is corroborated in table 6.

Table 6. Shelf life of tomato fruits treated with biocontrol agents adsorbed on chitosan-silica nanocomposites

11	10.00	a
12	10.75	a
7	11.25	ab
9	11.25	ab
14	13.25	abc
13	14.25	bc
10	14.75	c
15	15.25	c
1	24.25	d
16	24.25	d
2	24.50	d
5	25.25	d
8	25.50	d
3	26.00	d

4	27.25	d

Means linked with a similar letter are not significantly different. LSD $_{0.05}$

The increased shelf life observed in the study was attributed to antimicrobial and physiological effect of BCAs and chitosan-silica nanocomposites. For instance, use of protective cultures, particularly lactic acid bacteria (LAB), micro-organisms from indigenous flora and their antimicrobial products, have been used in preservation of cut-fresh produce. Thus the microbes have an effect against spoilage causing microbes. In vitro cultures of of most pathogenic microbes were inhibited by the beneficial microbial antagonists such as LAB, *Bacillus subtilis*, Effective micro-organisms among others. Combination of BCAs with essential oils like thyme and other materials with antimicrobial properties like chitosan, reduced the pathogenic agents significantly significantly ($P<0.05$).

BCAs induce the fruit texture, reducing the softening aspect. Softening in fruits is always associated with spoilage. The BCAs are also implicated to affect the production of ethylene. This is the fruit ripening hormone in climacteric fruits. Inhibition of production of the hormone results in delayed fruit ripening hence, increased shelf life.

In addition to BCAs effect, chitosan is known to have antimicrobial effects. The compound also has gel like properties, thus applied on fruits minimises moisture loss in fruits reducing wilting and also acts as pathogens. The enhanced inhibition was attributed to positive charges of amino groups that disrupted cellular functions of the charged bacterial membrane, thus destroying the cells. Interactions between positively charged chitosan molecules and negatively charged residues of bacterial cell surface also played an important role in the inhibitory effect of gram negative bacterial pathogen. The inhibition was also attributed to direct toxicity, chelation of nutrients and minerals from the pathogen. In addition, the biopolymer properties of chitosan engulfed the pathogen causing suffocation and denied it nutrition. Additionally, chitosan stimulated microbial degradation of *R. solanacearum* pathogen in a manner resembling the application of a hyper-parasite. Chitosan has also been found to inhibit other plant pathogens like *Pseudomonas syringe* resulting in reduced crop losses (Rodrigo *et al*., 2006).

Adsorption of BCAs on CISNC gel increased inhibition of the pathogen significantly ($P<0.05$). This was attributed to the inhibitory effect of individual members and the resultant synergy. Adsorption of BCAs was made possible by the fact that, most microbes have a net charge on their membranes, which allow their adsorption to polar materials.

3.5 Rhizosphere Water Conservation

Treatment of tomato plants in the greenhouse with Chitosan-silica nanocomposites and BCA-nanocomposites reduced the soil moisture loss known as increased water utilization efficiency significantly ($P<0.05$) compared to the controls. Adsorption of BCAs on chitosan-silica nanocomposites enhnanced their efficacy in soil-water conservation. The results of bionanocomposites treatment of tomato fruits is corroborated in table 7.

Table 7: Soil water loss in tomato plant rhizosphere treated with bionanocomposites

Treatment	Means of water loss in rhizosphere	
2	4.967	a
13	5.000	a
14	5.100	a
1	5.233	ab
3	5.600	abc
5	5.667	abc
17	5.700	abc
4	5.833	abc
15	5.900	abc
16	6.133	bcd
12	6.233	cd
11	6.900	d
7	9.733	e
9	10.200	ef
8	10.333	ef

10	11.000	fg
6	11.100	fg
18	11.43 g	
Se	0.1144	
Cv (%)	1.6	

Means linked with a similar letter are not significantly different. LSD $_{0.05}$

The reduced water loss in this study was attributed to effect of biocontrol agents and chitosan-silica nanocomposites. For instance, according to Lee *et al*., 1999, chitosan applications on plants, results in reduced transpiration due to reduced stomatal apertures. These authors demonstrated that chitosan inhibited light-induced opening of stomata in tomato and *Commelina communis* via inducing H_2O_2 production in the guard cells. The reported effects of chitosan on stomatal aperture suggest the possibility that chitosan might be a valuable antitranspirant with useful agricultural applications. Chitosan also has gel like properties which forms a matrix with the soil particles reducing water loss through evaporation or percolation.

Additionally, BCAs increases microbial activity in the soil. This increases biodegradation, mineralization, hydrolysis and other biotic activities which improves the soil structure. These activities increases the soil water holding capacity, thus increased water utilization efficiency (Lucas-Garcia *et al*., 2004.

Conclusion and Recommendations

The study emboldened the findings of a previous study, where adsorption of biocontrol agents on chitosan immobilized silica showed a resultant synergy by increasing resistance of tomato varieties against tomato bacterial wilt caused by *R. solanacearum.* The technology of delivering biocontrol agents by adsorbing them on chitosan silica nanocomposites, though new, holds a great promise in controlling the devastating and persistent pathogen under field and storage conditions. Use of chitosan and silica in the composites, enhances the tomato fruits shelf life therefore resulting in development of a production and post-harvest efficacious product. Thus, reduced value chain losses. The reduction of soil water loss by the bionanocomposites is a panacea for water conservation in greenhouse farming, an enterprise with a high demand for the scarce resource due to the high evapotranspiration.

Acknowledgements

The authors wish to express their gratitude to Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya for funding the research work.

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