academicJournals

Vol. 7(7), pp. 64-69, November 2015
DOI: 10.5897/JBR2015.0172
Article Number: 37CB90A56517
ISSN 2006-9871

http://www.academicjournals.org/JBR

African Journal of Bacteriology Research

Full Length Research Paper

In vitro efficacy of certain botanicals and their effect on some biochemical properties of *Pectobacterium carotovora* susp. *carotovora* inoculated tomato (*Lycopersicon esculentum* L.)

Sabiu Hussaini Adamu[1]* and Abhilasha A. Lal[2]

[1]Department of Crop Protection, Bayero University, Kano, P. M. B. 3011 Nigeria.
[2]Department of Plant Pathology, Allahabad School of Agriculture, SHIATS-Allahabad-211007, U. P. India.

Received 19 May, 2015; Accepted 11 August, 2015

Tomato (*Lycopersicon esculentum* L.) is a member of family Solanaceae. It is herbaceous, annual to perennial, prostrate and sexually propagated plant with bisexual flower. Tomatoes are attacked by many kinds of pathogens such as fungi, insects, nematodes, bacteria, viruses and viroid. Among bacterial diseases, bacterial soft rot devastates this important crop causing a huge decrease in yield and a greater loss in produce than any bacterial disease known. Yield losses due to post-harvest diseases of fruits and vegetables range from 20-30% but losses due to soft rot bacteria may reach up to 100% under insufficient conditions of storage facility, this have huge impacts on famers and vendors. *In vitro* efficacy of certain botanicals against bacterial soft rot of tomato were tested in the months of February to March, 2015 in the Department of Plant Pathology and Department of Biochemistry, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Deemed University)– Allahabad, UP, India. Four botanicals were evaluated against the bacterial soft rot of storage tomato at 2 and 8 days after inoculation. After evaluation of botanicals efficacy, their effect on some biochemical properties of the treated tomato such as reducing sugars, vitamin C and pH were also evaluated. Turmeric 30% (T_4) proved to be the best botanical to inhibit the bacterial growth while turmeric 20% (T_3) and neem 30% (T_6) appeared as superior in preserving biochemical properties of the tomato respectively.

Key words: Tomato, pathogens, bacterial disease.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is a member of family Solanaceae (Taylor, 1986; Rashid and Singh, 2000). It is herbaceous, annual to perennial, prostrate and sexually propagated plant with bisexual flower. It's typically day neutral plant and self-pollinated vegetable crop. Scientific information indicates that the cultivated tomato originated in a wild form in the Peru-Ecuado-Bolivia area of the Andes, that is, South America

*Corresponding author. E-mail: plptbuk2013@gmail.com

(Vavilov, 1951; Rick, 1969). Tomatoes are attacked by many kinds of plant pathogens such as fungi, insects,nematodes, bacteria, viruses and viroid. Among bacterial diseases, bacterial soft rot devastates many important crops particularly potato and tomatoes, causing a huge decrease in yield and a greater loss in produce than any bacterial disease known (Akbar et al., 2014). Although chemical treatment is effective, it has the problem of introducing resistance to bacterial pathogens of man or animal and reaching the pathogen which are well protected in vascular system, lenticels etc. and in this case even systemic bactericide failed when applied to postharvest as there is no vascular activity in harvested fruit or tuber (Czajkowski et al., 2011). Excessive use of chemicals for the control of postharvest plant diseases, have been counterproductive due to their residual effects, causing various problems in human, animals and environment (Nicholson, 2007). These problems caused by synthetic pesticides and their residues have increased the need for the search of effective biodegradable pesticides with greater selectivity (Al-Samarrai et al., 2012; Slusarenko et al., 2008).

The alternative strategies have included the search for new types of pesticides, which are often effective against a limited number of specific target species, are biodegradable into non-toxic products and suitable for use in integrated pest management programs (Al-Samarrai et al., 2012). Plant products effectively meet this criterion and have enormous potentials to influence modern agrochemical research. The use of botanicals or plant products is gaining popularity because they have been found to be non-toxic, more systemic with little mammalian toxicity (Bankole, 1996). Botanicals degrade more rapidly than most chemicals pesticides, and therefore are considered to be eco-friendly and less likely to kill beneficial pests than synthetic pesticides with longer environmental retention. Most botanicals degrade generally within a few days, and sometimes even within few hours (Siddiqui and Gulzar, 2003). Although botanicals have the potential of being safe agrochemicals but their effect on biochemical and physicochemical properties of vegetables have been poorly investigated. This work intends to study the effect of botanical extracts on some biochemical properties of treated tomato fruits.

MATERIALS AND METHODS

Source of materials used

The bacterial culture MMC-2112 (T) used in the experiment was procured from Microbial Culture Collection, National Centre for Cell Science.

Preparation of plant extracts (botanicals)

The aqueous plant extracts and their concentrations were prepared according to a method described by Obongoya et al. (2010), with minor modifications. Aqueous extracts of easily available plants in Allahabad as listed below were prepared for the experiment at two concentrations (20% and 30%) each:

T_1 -Ginger (*Zingiber officinale*) 20%, T_2 -Ginger (*Zingiber officinale*) 30%, T_3-Turmeric (*Curcuma longa*) 20%, T_4-Turmeric (*Curcuma longa*) 30%, T_5-Neem Seed (*Azadirachta indica*) 20%, T_6-Neem Seed (*Azadirachta indica*) 30%, T_7-Coriander (*Coriandrum sativum* L.) 20%, T_8-Coriander (*Coriandrum sativum* L.) 30%, T_{0a}-Sterile distilled water (Untreated Control), T_{0b}-Streptomycin sulphate (Treated control).

The plant materials were first oven dried (except neem seeds and turmeric which were dry) and grinded in to powder, using electric grinder (Mixer Grinder). Dried plant tissues (20 g/100 ml and 30 g/100 ml) were measured and soaked for 24 h in distilled water. Then suspension of each plant extract was filtered using 4 layer muslin cloth, 2 times. The plant extracts were then stored for later use in a refrigerator at 4°C. Discs of 12.7 mm were soaked in these extracts for 24 h and used as botanical treatment on the bacterial lawns under *in vitro*. While for streptomycin sulphate, only 1 g of powder was used in 100 ml sterile distilled water after which discs were soaked and used as above (positive control). In case of sterile distilled water (negative control), discs were just soaked and used as above.

Efficacy of four botanicals against bacterial soft rot of tomato fruits

Method described by Rahman et al. (2012a) was followed with little modifications. Efficacy of four botanical extracts was evaluated against bacterial soft rot of tomato fruits. The extracts were prepared as described above and each tomato fruit was drilled with 14 mm cork borer, then treated the cut section and it was covered with the plant extract for 30 min. Inoculum of the soft rot bacterium was prepared at concentration of 10^7 cfu/ml and 12.7 mm paper discs were soaked in for 30 min. The plant extract treated tomatoes were inoculated with the paper disc soaked in the inoculum suspensions by introducing a disc into a fruits section and replacing the cap. Inoculated tomatoes were then incubated in loosely tight polyethene bags at room temperature along with controls. Visual observations were made at 2 and 8 days after inoculation where data on disease incidence among fruits, followed by some biochemical properties of the fruits such as reducing sugars, Vitamin C and pH due to treatments been valuated. Disease incidence was determined after 2 and 8 days of inoculation (Plate 1) using the formula below:

$$\textit{Disease incidence} = \frac{\textit{Number of infected fruits}}{\textit{Total number of fruits}} \times 100$$

Estimation of ascorbic acid (Vitamin C): Volumetric Method described by Thimmaiah, (2009) was followed.

Estimation of reducing sugar by DNS Method: Standard method of DNS was followed.

Estimation of tomato fruit pH

pH is the measure of H^+ ion activity (concentration); it measures active acidity. Standard method of pH determination using pH meter was followed and fruits were first crushed (macerated) using pistil and mortar and then juice produced was tested using standardized pH meter which was standardized with 4 and 7 buffer solutions (Tilahun, 2013). After standardization 5 ml of the each tomato juice was diluted in 50 ml deionized water for the measurement.

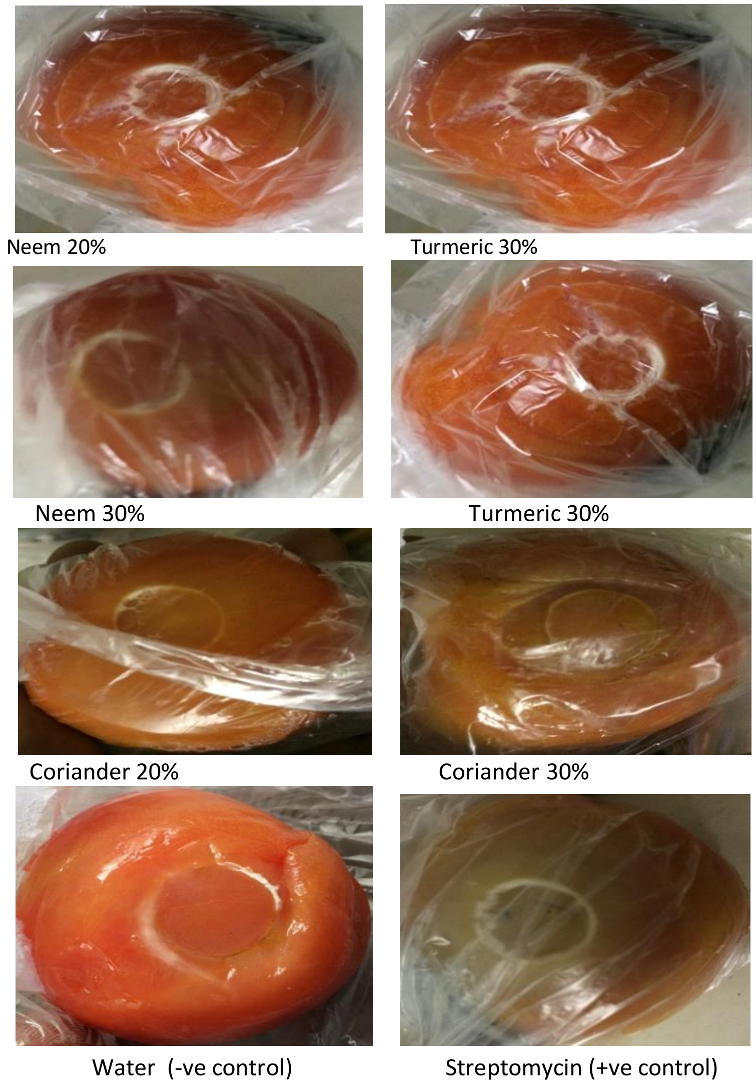

Plate 1. Disease incidence at eight days after inoculation for the best extracts and checks.

RESULTS AND DISCUSSION

Efficacy of selected botanicals against bacterial soft rot of storage tomato

Four botanicals were selected based on their performance as inhibition efficiency and efficacy under *in vitro* screening and evaluated against the bacterial soft rot of tomato under storage condition. Disease incidence/infection (%) was determined according to Rahman et al. (2012b). Among the botanicals evaluated turmeric 20% (T_3), turmeric 30% (T_4), neem 30% (T_6), coriander 20% (T_7) and coriander 30% (T_8) had the lowest disease incidence of 40% at two days after inoculation compared to rest of botanicals. The least mean value among the treatments was in treated control (T_{0b}) with 0% while the highest incidence was recorded with untreated control (T_{0a} =100%) (Table 1). The microbial activity shown by turmeric may be due to the action of its volatile oil constituent curcumin which has enolizable β-diketo group as chelating ligand (Rachana and Venugopalan, 2014) while antimicrobial activity of coriander may be attributed to its essential oil, it also known to exhibited bactericidal activity against most gram negative and gram positive bacteria (Silva et al., 2011). Its (coriander essential oil) act by membrane damage.

While incidences of the bacterial soft rot were observed in all ten treatments (that is, including treated and untreated control) at eight days after inoculation, this could be due to resurfacing and replication of resistant colonies of the inoculum. The incidence of the soft rot disease was found to be 100% in all the botanical treated fruits and untreated control while in treated control it was found to be 40% as depicted in Table 1. The microbial activity shown by turmeric may be due to the action of its volatile oil constituent curcumin which has

Table 1. Per cent disease incidence (soft rot of tomato) as affected by different treatments after two days of inoculation.

Treatments	(%) Disease Incidence after 2 days of inoculation	(%) Disease Incidence after 8 days of inoculation
T_1 (Ginger 20%)	60^{bc}	100^{bc}
T_2 (Ginger 30%)	60^{bc}	100^{a}
T_3 (Turmeric 20%)	40^{c}	100^{bc}
T_4 (Turmeric 30%)	40^{c}	100^{c}
T_5 (Neem Seed 20%)	80^{ab}	100^{ab}
T_6 (Neem Seed 30%)	40^{a}	100^{abc}
T_7 (Coriander 20%)	40^{a}	100^{abc}
T_8 (Coriander 20%)	40^{a}	100^{ab}
T_{0a}(Untreated Control)	100^{a}	100^{ab}
T_{0b} (Treated control)	00^{c}	40^{c}

All mean values with same letter(s) are statistically non-significant. Tab. t value (0.05) =1.81.

Table 2. Reducing sugars (%) of tomato fruit, as affected by different treatments.

Trt. Symbol	Treatment	Means Red. Sugar (%)
T_1	Ginger 20%	2.983^{de}
T_2	Ginger 30%	3.190^{bcd}
T_3	Turmeric 20%	3.430^{ab}
T_4	Turmeric 30%	3.340^{abc}
T_5	Neem 20%	3.343^{abc}
T_6	Neem 30%	3.446^{a}
T_7	Coriander 20%	3.023^{de}
T_8	Coriander 30%	3.130^{cde}
T_{0a}	Untreated Control	3.410^{ab}
T_{0b}	Treated control	3.380^{abc}
T_{0c}	Untreated un- inoculated control	2.890^{e}

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.357 CD (0.05) = 0.250. Tab.t value (0.05) =1.80. All mean values with same letter(s) are statistically non-significant.

enolizable β-diketo group as chelating ligand (Rachana and Venugopalan, 2014) while Slusarenko et al. (2008) reported neem to have active substance Azadirachtin which is under subclass of compound limonoids, class triterpenes and is active against a wide range of microbes and/or pests with up to 90% efficacy in most cases (Akbar et al., 2014; Koul and Walia, 2009). While the antimicrobial activity of coriander may be attributed to its essential oil, it also known to exhibited bactericidal activity against most against gram negative and gram positive bacteria (Silva et al., 2011). It (coriander essential oil) act by membrane damage (Silva et al., 2011).

Biochemical properties of tomato after *in vitro* evaluation

Reducing sugars

The highest reducing sugar content percent was recorded in neem 30% (T_6=3.45) then, turmeric 20% (T_3=3.43) followed by fresh tomato (T_{0a}=3.41), while treated control (T_{0c}=2.89) has the least mean followed by ginger 20% (T_1=2.98) as presented in Table 2. The result in general indicated the potential advantage of using these botanicals against the concerned pathogen as they do not hamper the fruits biochemical constituents.

The total reducing sugars content per cent of tomato fruits ranges between 1.7 to 4.7% (Yilmaz, 2001) and similar results were reported by Tadesse et al. (2012) with ranges between 2.728 – 3.561% at eight days of storage, while Hossain et al. (2010) with ranges between 2.36%-2.74% for various genotypes grown at Rangpur, Bangladesh. From the above information, it’s possible that most of the botanicals did not decrease the reducing sugars content of the fruits but rather a slight increase was recorded when compared to the three controls, which could imply an advantage in using them. But this view needs to be further studied as is first attempt its kind.

Table 3. Vitamin C (mg/100mg) of tomato fruits as affected by different treatments.

S.No	Treatment	Treatment Means
T_1	Ginger 20%	19.603^{cd}
T_2	Ginger 30%	20.100^{abc}
T_3	Turmeric 20%	20.400^{a}
T_4	Turmeric 30%	20.230^{ab}
T_5	Neem 20%	20.140^{ab}
T_6	Neem 30%	20.270^{ab}
T_7	Coriander 20%	19.496^{de}
T_8	Coriander 30%	19.876^{bcd}
T_{0a}	Untreated Control	20.176^{ab}
T_{0b}	Treated control	20.473^{a}
T_{0c}	Untreated un- inoculated control	18.993^{e}

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.701 CD (0.05) = 0.511. Tab.t value (0.05) = 1.80. All mean values with same letter(s) are statistically non-significant.

Table 4. pH of tomato fruits as affected by different treatments.

S.No	Treatment	pH means
T_1	Ginger 20%	4.353^{bc}
T_2	Ginger 30%	4.383^{bc}
T_3	Turmeric 20%	4.660^{a}
T_4	Turmeric 30%	4.473^{ab}
T_5	Neem 20%	4.670^{a}
T_6	Neem 30%	4.640^{a}
T_7	Coriander 20%	4.350^{bc}
T_8	Coriander 30%	4.180^{c}
T_{0a}	Untreated Control	3.916^{d}
T_{0b}	Treated control	3.886^{d}
T_{0c}	Untreated un- inoculated control	4.673^{a}

Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.281 CD (0.05) = 0.217. Tab.t value (0.05) = 1.80. All means with same letter(s) are statistically non-significant.

Ascorbic acid content of tomatoes

Vitamin C (mg) content of the tomatoes were also found to differ among the treatments only slightly but significant, the maximum mean value been recorded in untreated control (T_{0b}=20.47) followed by turmeric 20% (T_3=20.40), then neem 30% (T_6=20.27) while least mean value was found in treated control (T_{0c}=18.99) followed by coriander 20% (T_7=19.59) which is second to lowest (Table 3).

All the mean values were found to be within standard range as reported by Hossain et al. (2010), that vitamin C content of tomato varies from 17.32 mg/100 g to 26.59 mg/100 g. Similar result was also reported by Kallo (1985). Treatment means, botanicals and concentration were significant, but as this is an attempt to trace the effect of botanicals on some biochemical properties of tomato, it is suggested that further investigation should be done.

pH of tomatoes after eight days of inoculation

pH means values were found to differ significantly among the treatments. The highest pH mean value was in neem 20% (T_5=4.68), followed by treated control (T_{0c}=4.67) then turmeric 20% (T_3=4.66) while least pH mean value was in untreated control (T_{0b}=3.89), then untreated un-inoculated control (T_{0a}=3.92) followed by coriander 30% (T_8=4.18) (Table 4). The high pH in neem 20% (T_5=4.68) and treated control (T_{0c}=4.67) may be explained by the way it's active ingredients work, possibly is by rising pH to inhibit microbial growth as suggested by Czajkowski et al. (2011).

While low pH in untreated control can be due to the respiration of the tomato fruits, since it does not appear to be extremely low. The result presented (Table 4) here is also in agreement with several earlier reports which shows positive but irregular correlation between reducing sugars, pH, (Georgelis et al., 2004), pH, reducing sugars and vitamin C, and /or total soluble solids but negative with total acidity as reported by Tilahun (2013) that pH and reducing sugar content of harvested tomato fruits are affected by its maturity state and time taken respiring by fruit off plant. In general, the high pH, reducing sugars, and vitamin C mean values recorded in this experiment might be attributed to fact that the tomato was produced under organic system of farming or due to a slight increase due to treatments.

Conclusion

Significant results were obtained when the four botanicals were screened *in vitro*. In the present study turmeric 30% (T_4) proved to have highest potential to be used for the management of bacterial soft rot of tomato caused by *Pectobacterium carotovora* susp. *carotovora* compared to the rest botanicals, whereas neem 30% (T_6) appeared to maintain the highest fruit reducing sugars, compared to the other treatments. Turmeric 20% (T_3) appeared to maintain highest fruits' vitamin C value while fruit pH highest mean value was by neem 20% (T_5). Turmeric 30% can be best recommended fort the management of bacterial soft rot of tomato fruits while turmeric 20% and neem 30% can be used if biochemical properties of tomato genotype are so low and might be jeopardized. The results of the experiment are based on single trial conducted under Allahabad, UP, India.

Conflict of interests

The authors have not declared any conflict of interest.

REFERENCES

Akbar A, Din S, Ahmad M, Khan G, Alam S (2014). Effect of Phytobiocides in controlling soft rot of tomato. J. Nat. Sci. Res. 4(11): 2225-09921.

Al-Samarrai G, Singh H, Syarhabil M (2012). Evaluating eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides. Ann. Agric. Environ. Med. 19(4): 673-676.

Bankole SA (1996). Effect of essential oils from two Nigerian medicinal plants (*Azadirachta indica* and *Morinda lucida*) on growth and aflatoxin B_1 production in maize grain by a toxigenic *Aspergillus flavus.* Lett. Appl. Microbiol. 24(3): 190-192.

Czajkowski R, Perombelon MCM, Vanveen JA, Van der Wolf JM (2011). Control of blackleg and tuber soft rot of potato caused by Pectobacterium and Dickeya species: A Review. Plant Pathol. 10:1365-3059.

Georgelis N, Scott JW, Baldwin EA (2004). Relationship of tomato fruit sugar concentration with physical and chemical traits and linkage of RAPD markers. J. Am. Soc. Hortic. Sci. 129(6): 839-845.

Hossain ME, Alam MJ, Amanullah ASM (2010). An assessment of physicochemical properties of some tomato genotypes and varieties grown at Ranghpur. Bangladesh Res. Public J. 4: 235-243.

Kallo G (1985). Tomato. Published by R.H. Saehdev for Allied Publishers Pvt. Ltd. India. P 33.

Nicholson GM (2007). Fighting the global pest problem: preface to the special Toxicant issues on insecticidal toxins and their potential for insect pest control. Toxicon 49(4):413-422.

Obongoya BO, Wagai SO, Odhiambo G (2010). Phytotoxic effect of selected crude plant extracts on soil-borne fungi of common bean. Afr. Crop Sci. J. 18(1):15-22.

Rachana S, Venugopalan P (2014). Antioxidant and bactericidal activity of wild turmeric extracts. J. Pharmacogn. Phytochem. 2(6):89-94*.*

Rahman MM, Eaqub AM, Khan AA, Hashim U, Akanda AM, Hakim MA (2012a). Characterization and identification of soft rot bacterial pathogens in Bangladeshi potatoes. Afr. J. Microbiol. Res: 6(7):1437-1445.

Rahman MM, Khan AA, Ali ME, Mian IH, Akanda AM, Abd Hamid SB (2012b). Botanicals to control soft rot bacteria of potato. Sci. World J. 2012:796472.

Rashid MA, Singh DP (2000). A manual on vegetable seed production in Bangladesh. AVRDC-USDA, Bangladesh project. Available at: http://203.64.245.61/web_docs/manuals/bangladesh_seed_production.pdf.

Rick CM (1969).Origin of cultivated tomato, current status of the problems, Abstract xi of Internal Botany Congress P 180.

Siddiqui FA, Gulzar T (2003). Tetracyclin triterpenoids from the leaves of *Azadirachta indica* and their insecticidal activities. Chem. Pharm. Bull. (Tokyo) 51:415-417.

Silva F, Ferreira S, Qeiroz JA, Dominques FC (2011). Coriander (Coriandrum sativum L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry. J. Med. Microbiol. 14: 79-86.

Tadesse T, Seyounm TW, Woldestsadik K (2012). Effect of varieties on changes in sugar content and marketability of tomato stored under ambient condition. Afr. J. Agric. Res. 4(14): 2124-2130.

Taylor IB (1986). Biosystematics of the tomato in the tomato crop: A scientific basis for improvement (eds. Artherton, J.G, and Rudich. J.)

Thimmaiah SR (2009). Standard Methods of Biochemical Analysis; Kalyani Publishers ©1999, Printed (2009). ISBN; 81-7663-067-5 .51-278.

Tilahun AT (2013). Analysis of the effect of maturity stage on the postharvest biochemical quality characteristics of tomato (*Lycopersicon esculentum* Mill*.).* Int. J. Pharm. Appl. Sci. 395: 180-186.

Vavilov NI (1951).The origin, variation, immunity and breeding of cultivated plants. Chronica. Bot. 13:1-366.

Yilmaz E (2001). The chemistry of fresh tomato flavor. Turk. J. Agric. 25:149-155.