

IN VITRO EVALUATION OF DIFFERENT LOCALLY AVAILABLE PRESERVATIVES AGAINST THE FUNGUS CAUSING TOMATO FRUIT ROT DISEASE

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ABSTRACT

Received 28. 5. 2020 Revised 14. 6. 2021 Accepted 29. 6. 2021 Published 1. 12. 2021

ARTICLE INFO

Regular article

The post-harvest tomato fruit rot caused by Alternaria solani is a major threat to the tomato fruits. Our main objective of this study was to assess the locally available plant based preservatives against post-harvest tomato fruit rot. The in-vitro antifungal effect of 09 different treatments (07 plants based = black pepper, red pepper, turmeric, clove, garlic, onion and papaya seed and 02 chemical preservatives = Potassium metabi-sulphate, PMS and Sodium benzoate, SB) showed significant (P < 0.05 = 0.0000) variation among the treatment groups. Black pepper followed by red pepper and turmeric remained the best antifungal and preservative after 05, 10 and 15 days after treatment (DAT) in B-group (inoculated and uninjured fruits). However, in A-group (inoculated and injured fruits), lowest infection percent was recorded for black pepper followed by red pepper and turmeric after 05, 10 and 15 DAT. The response of all treatments was obvious after 15 DAT. No infection of test fungus was recorded when black pepper and red pepper were applied. Whereas, with the application of turmeric (8.33%) and garlic (9.33%) lowest infection percent was observed with no significant difference. The response of onion (4.33%), papaya seed (6%), clove (7.66%) and SB (15%) appeared moderate with no significant difference. After 30 days, the lowest number of spoilt fruits were recorded with the treatment of black pepper (01) and red pepper (01) followed by turmeric (02), clove (02), onion (02) and SB (02) in B-group. Based on best physical structure, maximum numbers of tomato fruits were observed in clove (04) followed by black pepper (03) and SB (03) when treated without any injury (B group). While in case of A group, almost all fruits in all treatments become spoilt after 30 days of treatment. It has been proved that locally available plant based preservatives have an excellent antifungal and preservative potential against post-harvest tomato fruit rot disease caused by Alternaria solani. These plant based preservatives increase the shelf-life of tomatoes without any health hazards. To the best of our knowledge, this is the first time conducted study, where antifungal and preservative potential of locally available plant products against post-harvest tomato fruit rot caused by Alternaria solani have been explored.

Keywords: Antifungal effect; preservatives; botanicals; tomato fruit rot

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is considered as one of the most important kitchen garden vegetable plant. It is also cultivated in large acreage for commercial purpose and requires relatively little space for large production (**Nasir et al., 2015**). It belongs to the genus *Lycopersicon* and family Solanaceae. It is herbaceous sprawling plant growing to 1-3 m in height with weak woody stem. Generally it is categorized as a vegetable, but in botanical terms, the tomato is the fruit born on a vine. The special nutritive values of tomato make it most important protective foods for humans (**Raiola et al., 2014**). It supplies essential vitamins and minerals to the diet, which are necessary to maintain good health. Tomato has a great importance as play an important role in variety of food production and make food appetizing (**Mbuk et al., 2011**). Further, the fruit has medicinal value as a gentle stimulant for kidneys, and washing off toxins that contaminate the body systems. It improves the status of dietary anti-oxidants (lycopene, ascorbic acid and phenols) in diet. For intestinal and liver disorders, tomato juice is known to be effective (**Raiola et al., 2014**).

Beside nutritional and economic importance of tomatoes, several biotic and abiotic factors are responsible for decline in the yield. Tomato is considered as one of the most susceptible vegetable crop causing 30 to 40% yield losses in tropical regions. It is highly perishable crop and has been shown that as high as 50% of these products are lost between rural production and consumption in the tropical areas. The major problems include improper management, insect pests and diseases during pre-and post-harvest stages, use of poor seeds due to un-

availability of standard seeds etc (Charchar et al., 2003). The yield of tomato is also affected by number of pathogenic diseases every year. Those diseases are caused by different kinds of fungi, bacteria, viruses and nematodes. Generally, such diseases are develop through soil-borne, above-ground infections and in some instances, are transmitted through insect feeding. There are numerous micro-organisms that cause post-harvest decay of tomatoes. Among these, fungi and bacteria are the most destructive (Chohan et al., 2016). However, tomato fruit rots are mainly caused by fungi are more important and needs special consideration. Studies reveal that several fungal species are associated to cause fungal infections in tomatoes such as *Geotrichum candidum*, *Rhizopus* stolonifer, black mould rot caused by *Alternaria* sp., Fusarium rot by *Fusarium* sp., are more commonly occurring diseases (**Mujib** et al., 2007).

https://doi.org/10.15414/jmbfs.3188

In tomato fruits, Alternaria rot has been reported as the most common and severe diseases. It may causes heavy losses in quality and quantity of the fruits, therefore, rendering huge number of tomato fruits unfit for consumers (Saeed et al., 2010). Alternaria is the main decay causing fungus of post harvest tomato fruits while responsible for black rot lesions on tomato fruits. Alternaria species are major plant pathogens, which cause at least 20% of agricultural spoilage; most severe losses may reach up to 80% of yield, affecting the leaves, stems, flowers and fruits (Nowicki et al., 2012). Recently, Sajad et al. (2017) reported that tomato fruits affered by fruit rot diseases are mainly caused by Aspergillus niger, Aspergillus flavus, Alternaria alternata, Alternaria solani, Geotrichum candidum, Mucor racemosus, Fusarium oxysporum, Fusarium moniliforme, Penicillium digitatum, Rhizopus stolonifer, Alternaria alternata, Colletotrichum

lycopersici, Sclerotium rolfsii, Myrothecium roridum, Phoma destructiva and *Trichothecium roseum.* Percentage frequency of occurrences on all tomato fruits were found maximum for *Alternaria alternata* 16.51%. However, control of tomato fruit rots always remained challenge for researchers, thus it is difficult to harvest tomatoes without causing any damage. Moreover, fungal rots on tomatoes are not only problem in Pakistan; nevertheless, is a worldwide threat.

The influence of food preservatives for increasing the shelf life of fruits and vegetables are well documented in the literature. However, a very limited focus has been given to the preservatives, especially the locally available, for their antimicrobial activities against pathogenic organism particularly for post harvest fungi. The literature available on the disease indicated that management of post harvest fruit rot is still a big challenge for the researchers. Keeping in view the losses caused by the disease and various health hazards due to judicious use of pesticides, the present research work was planned to assess some preservatives for antifungal potential against fruit rot causing fungus in tomato fruits under *in vitro* conditions.

MATERIALS AND METHODS

Study location and materials

The present study was conducted in the laboratories of Department of Plant Protection, Sindh Agriculture University and surrounding fields of Tandojam, Pakistan. The *in vitro* antifungal potential of 09 different treatments including 07 plants based locally available preservatives and 02 chemical preservatives used to treat the tomato fruits in order to avoid the post-harvest rot causing fungi and increase the shelf life. The plant based materials like some vegetables and spices except papaya seed including black pepper, red pepper, turmeric, clove, garlic and onion were tested against Alternaria post-harvest rot of tomato by dividing into two groups; A-group (inoculated and injured tomato fruits); B-group (Inoculated and un-injured tomato fruits). In addition, two commonly used preservatives, Potassium metabi-sulphate (PMS) and Sodium benzoate (SB) were also tested for antifungal potential (Table 1, figure 1).

 Table 1 List of different preservatives used for *in vitro* antifungal potential against tomato post-harvest rot causing fungus

Treatment No.	Local Name	Botanical Name Piper nigrum		
T-1.	Black pepper			
T-2.	Red pepper	Capsicum annuum L.		
T-3.	Turmeric	Curcuma longa		
T-4.	Clove	Syzygium aromaticum		
T-5.	Papaya seed	Carica papaya L.		
T-6.	Garlic	Allium sativum		
T-7.	Onion	Allium cepa		
T-8.	Potassium metabi-sulphate (PMS)	-		
T-9.	Sodium benzoate (SB)	-		
T-10.	Control	-		

In vitro preservative and antifungal tests

Different locally available plant based and chemical preservatives were assessed against the fungus causing post-harvest fruit rot of tomato under in vitro in order to determine the antifungal potential in addition to preventives. Fresh and sound red colored tomato fruits, without any mechanical injury and crakes were purchased from a local market of Tandojam. All tomatoes were graded based on equal size and color and then thoroughly washed with tap water. Tomato fruits were categorized into two groups based on the intentionally provided mechanical injury (Group-A) and uninjured on the surface (Group-B). Mechanical injuries were provided with the help of sterilized needle. Both groups were inoculated with fruit rot causing fungus by dipping in suspension contained 4.6×10^7 conidia mL¹ of A. solani. adjusted by using haemocytometer under microscope and then kept for 10 minutes. A total of 06 inoculated fruits (replications) were dipped into the 10 percent preservative suspension of each for 05 minutes in order to test the antimicrobial effect of treatments. All the treated tomatoes were gently transferred to the plastic containers and then quickly wrapped with transparent polythen bags. Plastic containers were already disinfected with alcohol. Inoculated and untreated tomato fruits were kept as control. The experiment was laid out in randomized complete design (RCD) with six replications. All the treatments were incubated at room temperature.

Preparation of inoculums

The inoculum of fruit rot causing fungus was prepared from fresh fungal culture of *A. solani*. One half of culture plate was provided about 10 mL sterile distilled water and then brushed the culture surface. Suspension then transferred into beaker and water was adjusted up to 100 mL. The suspension was filtered through two layers of muslin cloth and filtrate was adjusted to 4.6×10^7 conidia

 mL^1 using haemocytometer under microscope. The resulting suspension was used for inoculation of healthy tomato fruits.

Preparation of stock solution

Fresh bulbs, seeds and other plant parts of each locally available plant based preservatives were thoroughly washed, chopped and grinded (figure 1, Table 1). In case of already powdered materials, they were used directly for the extraction of aqueous extract. A total of 50g of each plant based preservatives except chemical preservative was separately thoroughly macerated in grinder with 250mL of distilled water. The grinder was cleaned with tap water and then alcohol between operations for materials. The extracts kept for 18hrs and then were filtered through two layers of muslin cloth. All the plant based preservatives like as Potassium metabi-sulphate and Sodium benzoate were used 1 g per 100 mL (01%) distilled sterilized water.

T-1= Black pepper (Piper nigrum)	T-2= Red pepper (<i>Capsicum annuum</i> L.)	T-3= Turmeric (<i>Curcuma longa</i>)
T-4= Clove (Syzygium aromaticum)	T-5 = Papaya seed (<i>Carica papaya</i> L.)	T-6= Garlic (Allium sativum L.)
T-7= Onion (Allium cepa L.)	T-8 = PMS Potassium metabi- sulphate	T-9 = SB Sodium benzoate

Figure 1 Different botanicals used for *in vitro* management of fruit rot causing fungi in current experiments

Observations

After conducting experiment, data was recorded on daily basis, and colonies and infection of fungus were observed. In addition, temperature data was also recorded on daily basis. In the Group-A (mechanically injured), infection by *A. solani.* started earlier (5 days after inoculation) and progress very rapidly. About 5 days later of inoculation, slightly sunken spots with a faded blackish center were observed on Group-A tomato at injured sites. The superficial colonies growths of inoculated fungus were counted on daily basis. However, in case of uninjured (Group-B), the symptoms were noticed after 7-10 days of inoculation. In addition to test fungus, other saprophytic were also observed and counted. The fully spoilt tomato fruits were discarded and counted, whenever noticed, in the experiment. The experiment was ended over when all fruits become spoilt in both controls, Group A and B. At the end, non-infected and useable tomatoes were counted in order to compare the preservative and antimicrobial activity of plant based treatments. To estimate the percent fruit rot caused by fungi, a total of 20

fruits from each farmer's field, 10 from each broker of vegetable market and all available injured/rotted samples from each small shopkeeper were randomly observed based on the visual symptoms produced by fruit rot causing fungi. The disease intensity was calculated by using the formula of diseases incidence as under:

$$Percentage of Infected Fruits = \frac{Number of Infected Fruits}{Total Number of Fruits} X100$$

Statistical analysis

The data obtained in present study was statistically analyzed by using the standard procedures for analysis of variance, ANOVA (linear model), and mean separation (least significant difference, LSD) of all parameters including frequency (%), fruit infection (%) were analyzed using the computer software Statistix 8.1 (Analytical Software, 2005). All differences described in the text were significant at the 5% level of probability.

RESULTS AND DISCUSSION

In vitro antifungal effect of different preservatives

The analysis of variance for all treatments and methods (groups) used for antifungal potential against *A. solani* showed significant differences (P < 0.05 = 0.0000). All tested plant based and chemical preservatives showed antifungal potential. After 05 days of treatment, no any fruit rot and colony of test fungus was noticed in B-group (inoculated and uninjured fruits) in all treatments including control. Whereas, in A-group (inoculated and injured fruits), lowest infection percent was recorded for black pepper (2%) followed by red pepper (3.33%) and turmeric (3.33%); however, no significant difference was recorded among these three treatments. The maximum infection percent after 5 DAT was noticed in control (19.83%), PMS (16.50%), Onion (15.16%) and papaya seed (14.33%). The response of Garlic (8%), SB (12%) and Clove (11.83%) remained moderate (Table 2).

	Percent Infected Fruit											
Treatments Black pepper		05 D	AT		10 DAT				15 DAT			
	A-group		B-group		A-group		B-group		A-group		B-group	
	2	e	0	e	4.33	de	0	e	12.33	efg	0	g
Red pepper	3.33	de	0	e	6.33	de	0	e	17.67	efg	0	g
Turmeric	3.33	de	0	e	6.67	de	0	e	22	ef	8.33	fg
Garlic	8	cd	0	e	10.83	cd	0	e	32.33	de	9.33	fg
SB	12	bc	0	e	15.33	bc	2	e	45.33	cd	15	efg
Clove	11.83	bc	0	e	15.83	bc	1.5	e	47	cd	7.67	fg
Onion	15.17	ab	0	e	18	ab	2.67	e	52.33	bcd	4.33	fg
Papapya seed	14.33	b	0	e	18.33	ab	2.333	e	55	bc	6	fg
PMS	16.5	ab	0	e	20.5	ab	3.667	e	60	bc	24.33	ef
Control	19.83	а	0	e	24	а	21.17	ab	91.67	а	68	b
SE	2.7593				3.3776				10.523			
LSD	5.4779					6.7	/053		20.89			

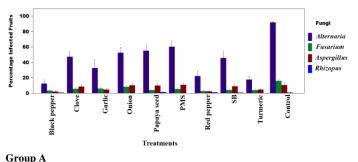
DAT = Days After Treatment, A-group = Inoculated and injured, B-group = Inoculated and un-injured.

After 10 days of treatment, colonies of test fungus were noticed in some treatments of B-group such as SB (2%), clove (1.5%), onion (2.66%), papaya seed (2.33%) and PMS (3.66); however, no significant difference was recorded among these treatments. Whereas, significantly higher infection percent was recorded in control (21.16%). In A-group, lowest infection percent was recorded for black pepper (4.33%) followed by red pepper (6.33%) and turmeric (6.66%) with no significant difference. The maximum infection percent after 10 DAT was noticed in control (24%) followed by PMS (20.5%), papaya seed (18.33%) and onion (18%). The response of Garlic (10.83%), SB (15.33%) and clove (15.83%) remained moderate with no significant difference (Table 2). The response of all treatments become obvious after 15 days of treatment, infection of test fungus was noticed in all treatment of B-group except black pepper and red pepper. No infection of test fungus was recorded in both black pepper and red pepper, showed greatest response as antifungal against Alternaria tomato fruit rot. Whereas, lowest percent fruit infected by test fungus was recorded for turmeric (8.33%) and garlic (9.33%) with no significant difference. Significantly higher infection percent was recorded in control (68%) and PMS (24.33%). The response of onion (4.33%), papaya seed (6%), clove (7.66%) and SB (15%) remained moderate with no significant difference. In A-group, significantly lowest infection percent of test fungus was recorded for black pepper black pepper (12.33%) followed by red pepper (17.66%) and turmeric (22%) with no significant difference. The maximum infection percent after 15 DAT was noticed in control (91.66%) followed by PMS (60%), papaya seed (55%) and onion (52.33%). The response of garlic (32.33%), SB (45.33%) and clove (47%) remained moderate with no significant difference (Table 2). In addition to test fungi, A. solani, some other post-harvest fruit rot fungi such as Aspergillus niger, Fusarium oxysporum and Rhizopus stolonifer were also appeared in different treatments after 15 days of treatment in both group A and B. The percent fruit infected by these fungi are also presented in figure 2. In B-group, no infection of test fungus as well as other fungi was recorded for both black pepper and red pepper, showed greatest response as antifungal against post-harvest fruit rot. In case of A-group, lowest percent fruit infected by test fungus and other fungi was recorded for black pepper and red pepper.

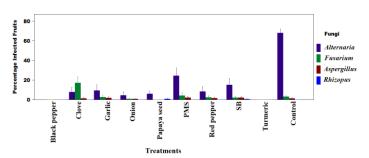
In vitro antifungal effect of different preservatives on the shelf life of tomato

The *in vitro* effect of different preservatives on the shelf life of tomato inoculated with *Alternaria solani* also showed great differences among different treatments. All tested plant based and chemical preservatives showed antifungal potential. However, at the same time also increased the shelf life of tomato. After 20 days of treatment, no any fruit become spoilt while treated the black pepper, red pepper, turmeric, clove onion and SB in A group. However, in B group, black pepper, red pepper and clove performed better than others. After 30 days, the

performance of all the treatments to increase the shelf life of tomato fruits becomes obvious. The lowest number of spoilt fruits was recorded in black pepper (01) and red pepper (01) followed by turmeric (02), clove (02), onion (02) and SB (02) in B group. However, the maximum number of physical best structure of tomato fruits were observed in clove (04) followed black pepper (03) and SB (03) when treated without any injury (B group). While in case of A group, almost all fruits in all treatments become spoilt after 30 days of treatment (Table 3, figure 3, 4).



SE = 6.7054, LSD (*P*<0.05) = 13.224



Group B

SE = 5.094, LSD (*P*<0.05) = 10.046

Note: A-group = Inoculated and injured; B) Inoculated and un-injured

Figure 2 Association of other fruit rot causing fungi observed during the period of whole experiment

Table 3 In vitro effect of different preservatives on the shelf life of tomato inoculated with Alternaria solani

Transformente		A-group			B-group			
Treatments	20 DAT			20 DAT	25 DAT	30 DAT	Physical Structu Remarks	
			Spoilt Tom	ato Fruits				
Black pepper	0	0	5	0	0	1	Spoilt	
Red pepper	0	0	6	0	0	1	Spoilt	
Turmeric	1	2	6	0	1	2	Spoilt	
Clove	0		6	0	1	2	Spoilt	
Papaya seed	2	4	6	2	3	5	Spoilt	
Garlic	1	3	6	1	2	5	Spoilt	
Onion	3	4	5	0	1	2	Spoilt	
PMS	2	4	6	2	3	5	Spoilt	
SB	2	3	4	0	1	3	Spoilt	
Control	2	5	6	3	6	6	Spoilt	
Mean	1.3000	2.60	5.600	0.8000	1.80	3.10		
SE	0.335	0.5617	0.2211	0.359	0.5735	0.6403		
LSD	0.0037	0.0012	0.0000	0.0528	0.0120	0.0009		
			Preserved To	mato Fruits				
Black pepper	6	6	1	6	6	5	3B	
Red pepper	6	6	0	6	6	5	1B	
Turmeric	5	4	0	6	5	4	2B	
Clove	6	5	0	6	5	4	4 B	
Papaya seed	4	2	0	4	3	1	Spoilt	
Garlic	5	3	0	5	4	1	1 B	
Onion	3	2	1	6	5	4	2B	
PMS	4	2	0	4	3	1	Spoilt	
SB	4	3	2	6	5	3	3 B	
Control	4	1	0	3	0	0	Spoilt	
Mean	4.70	3.40	0.400	5.20	4.200	2.900		
SE	0.335	0.5617	0.2211	0.359	0.5735	0.6403		
LSD	0.0000	0.0002	0.1039	0.0000	0.0000	0.0014		

DAT = Days After Treatment, B = Best physical structure, A-group = Inoculated and injured, B-group = Inoculated and un-injured.

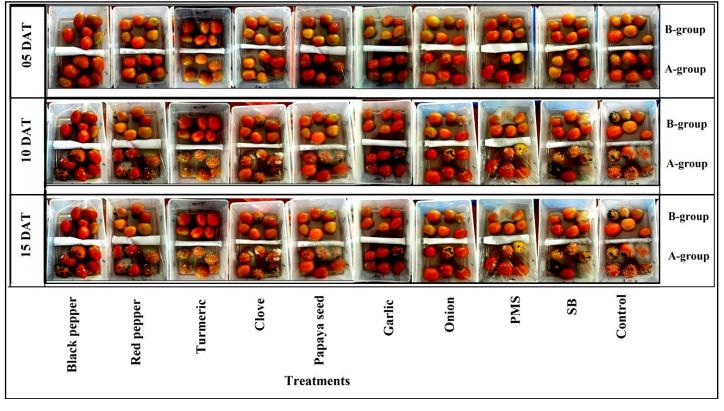


Figure 3 In vitro performance of different preservatives used as antifungal against A. solani

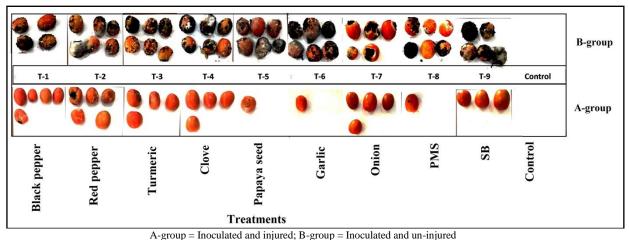


Figure 4 Spoilt and sustained tomato fruits after 25 days of treatments

The influence of food preservatives for increasing the shelf life of fruits and vegetables are well documented in the literature. However, a very limited focus has been given to the preservatives, especially locally available, for their antimicrobial activities against pathogenic organism particularly for post-harvest fungi. In the current study, the in vitro antifungal potential of 09 different treatments including 07 plants based locally available preservatives and 02 chemical preservatives used to treat the tomato fruits in order to avoid the post harvest rot causing fungi and increase the shelf-life. The plant based materials like as some vegetables and spices except papaya seed including black pepper, red pepper, turmeric, clove, garlic and onion were tested against Alternaria post harvest rot of tomato by dividing into two groups; A-group (inoculated and injured tomato fruits); B-group (Inoculated and un-injured tomato fruits). In addition, two commonly used preservatives, Potassium metabi-sulphate (PMS) and Sodium benzoate (SB) were also tested for antifungal potential. All tested plant based and chemical preservatives showed antifungal potential. After 05 days of treatment, no any fruit rot and colony of test fungus was noticed in Bgroup (inoculated and uninjured fruits) in all treatments including control. Whereas, in A-group (inoculated and injured fruits), lowest infection percent was recorded for black pepper, red pepper and turmeric with no significant difference. After 10 days of treatment, colonies of test fungus were noticed in some treatments of B-group such as SB, clove, onion, papaya seed and PMS; however, in A-group, lowest infection percent was recorded for black pepper, red pepper and turmeric with no significant difference. The maximum infection percent after 10 DAT was noticed in control followed by PMS, papaya seed and onion. The response of garlic, SB and clove remained moderate. Moreover, the response of all treatments become obvious after 15 days of treatment, infection of test fungus was noticed in all treatment of B-group except black pepper and red pepper. No infection of test fungus was recorded in both black pepper and red pepper that showed greatest response as antifungal against Alternaria tomato fruit rot. Whereas, lowest percent fruit infected by test fungus was recorded for turmeric and garlic with no significant difference. Significantly higher infection percent was recorded in control and PMS. In A-group, significantly lowest infection percent of test fungus was recorded for black pepper black pepper, red pepper and turmeric with no significant difference. In the recent past, Shampa et al. (2015) investigated the effect of chemical preservatives and storage conditions on tomato pulp by using various concentrations of sodium benzoate (0.05 and 0.1%) and potassium metabi-sulphate (0.05 and 0.1%). Our studies also used the same chemical preservatives and got the satisfactory results particularly as preservative but the antifungal activities of these chemicals remained moderate. However, the lowest number of spoilt fruits was recorded in black pepper, turmeric, clove, onion and SB. Based on the physical best structure, maximum numbers of tomato fruits were observed in clove followed by black pepper and SB when treated without any injury (A group). While in case of B group, almost all fruits in all treatments become spoilt after 30 days of treatment. In one study Olaleye et al. (2014) used some natural products to extend the shelf-life of perishable vegetables. The preservative effects of four plant extracts-Xylopia aethiopica, Piper nigrum, Tetrapleura tetraptera and Carica papaya seeds on tomato and pepper showed that plants can serve as good ethnomedicinal and potent sources of natural preservatives. Consistence to our study, Adekalu et al. (2009) conducted study to develop a method for prolonging the shelf-life of ground fresh tomato using fresh Allium sativum and Eugenia aromatica, as antimicrobial and preservative agent. Both A. sativum (Garlic) and E. aromatica (Clove) increased the shelf life of fresh tomato puree for a maximum of ten days. However, all above mentioned studies only used to increase the shelf-life but they did not focused for the antimicrobial activities of these plants against fruit rot diseases. In our study, we used 07 different kinds of plant based extracts and 02 chemical preventives that were not used before in any reported studies. In our study we found that these plants may have potential chemical compounds that need to be explored in deep by using advanced tools. Some plany based extracts such as black pepper followed by red pepper and turmeric were remained well in our study need to be analysed through HPLC for the extraction of possible chemical compound and their possible structural formula in ordert to further specifically analysed. No doubt, the use of fungicides is believed to be one of the most effective method, may help in reducing disease spread, if applied to greenhouse and tunnels in addition to field (**Roy et al., 2019**). Though all kind of pesticides and chemical preservative may have satisfactory results but are health hazardous and dangerous to the ecosystem. Thus current study suggests the use of different plant based preservatives as antifungal as well for the management of tomato post-harvest fruit rot.

CONCLUSION

Current study concludes that black pepper, red pepper and turmeric possess an excellent antifungal and preservative potential against post-harvest tomato fruit rot disease caused by *Alternaria solani*. These plant based preservatives suppress the growth of disease pathogen and increase the shelf-life of tomatoes without any health hazards. To the best of our knowledge, this is the first time conducted study, where antifungal and preservative potential of locally available plant products against post-harvest tomato fruit rot caused by *Alternaria solani* have been explored. Based on novel findings of our study we recommend the use of these locally available plant based preservatives against post-harvest tomato fruit rot disease caused by *Alternaria solani*.

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