Microbial association with harvested tomato fruits and its characterization

A
Thesis submitted to the
Odisha University of Agriculture and Technology Bhubaneswar
In partial fulfilment of the requirements for the Degree
of
Master of Science in Agriculture
(Plant Pathology)

By

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BHUBANESWAR-751003, ODISHA

2021

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CERTIFICATE-I

(a) This is to certify that the thesis entitled **"Microbial association with harvested tomato fruits and its characterization"** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** to the Odisha University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Mausumi Das, Adm. No. 191222201** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the courses of investigation has been duly acknowledged.

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CHAIRMAN
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CERTIFICATE-II

This is to certify that the thesis entitled **"Microbial association with harvested tomato fruits and its characterization"** submitted by **Mausumi Das, Adm. No. 191222201** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** has been approved by the student's advisory committee and the external examiner.

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ACKNOWLEDGEMENT

A few words will never be enough to express my gratitude to all who helped and supported me in completing this research. Only by our combined efforts, not my own, has the work assigned to me become a reality. With the help of Good Hearts, I was able to bring this cost of work to light. I shall like to pen down my immense gratitude for all those who directly or indirectly helped me in this endeavour.

With an overwhelming sense of legitimate pride and genuine obligation, I seize this rare opportunity to express my deep sense of gratitude, indebtedness and personal regards to my condign and acuminous advisor to Dr. Gayatri Biswal, Professor, Dept. of Plant Pathology, OUAT, Bhubaneswar, my Guide and Chairman of Advisory Committee for laying out the guidelines of research work and framing my mind to think systematically and logically. I have real appreciation and regard for her keen interest, scholarly and judicious guidance, whole hearted ever available and untiring help, painstaking effort and above all, the sense of forgiveness throughout my association with him. My working experience under him will always remain as an unforgettable experience of my life.

With great reverence I express my deep sense of gratitude for the pivoted role played by the amiable Members of my Advisory Committee, Dr. A K Senapati, Professor and Head, Department of Plant Pathology, OUAT, Bhubaneswar. Dr. G S Sahu, Professor and Head, Dept. of Vegetable Science, OUAT, Bhubaneswar, for their encouragement, constructive suggestions and valuable advice.

I feel greatly indebted and convey my profound sense of gratitude to my esteemed teachers and other staff members of Department of Plant Pathology, Dr. S K Beura Professor and Head (Retd.), Department of Plant Pathology, OUAT, Dr. S S Mahapatra, Professor, Dept. of Plant Pathology and Dr. M K Mishra, Professor, Dept. of Plant Pathology for their precious help, sincere cooperation and active support during my experiment for which I must thank all of them sincerely.

I do express my sincere thanks to Sashi maam and other staffs of Plant Pathology for extending their helping hand for the completion of my lab. work.

I also wish my sincere and heartfelt thanks to my classmates Puja, Suraj, Lipika, Debasmita and all my department seniors and juniors for their selfless, goodwill and moral support.

I would like to give special thanks to my friends Bijayalakshmi, Subhalaxmi, Subhashree, Ayushee, Sidhartha, Gyanisha Didi for warm alliance, ceaseless inspiration ever ready cooperation and constant moral support, mellifluous love, poignant affection, unsolicited assistance.

I express my heartfelt gratitude and thanks to my seniors Suchi didi, Swagatika didi for their timely cooperation, help and support in M.Sc. period.

Every effort is motivated by ambition and all ambitions have an inspiration behind. Words are not enough to express my whole-hearted and affectionate gratitude to my beloved parents Mr. Bikram Kishor Das and Mrs. Sarashi Das who have always been a source of inspiration for me because of their boundless love, unwavering affection, and unwavering constant encouragement throughout my educational career and companionship in my personal and professional life, without whose invaluable moral support and benevolence the thesis would not have seen the light of day. It is the time to surface out my affectionate gratitude to my family members whose everlasting love, encouragement and inspiration were my strongest asset during the course of my life with whose I achieved this level of education.

Lastly, I bow my head before The Almighty Lord Jagannath whose constant blessings enabled me to complete this research work successfully and give me courage to stand all.

Great thanks to all.

Bhubaneswar **(Mausumi Das)**

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ABBREVIATIONS

%	:	Percent
^{0}	:	Degree
^{0}C	:	Degree Celsius
a.i.	:	Active ingredient
@	:	At the rate of
CD at 1%	:	Critical difference with significance level of 1%
et al.	:	et alia (Co- workers)
Fig.	:	Figure
i.e.	:	That is
No.	:	Number
SE (±)	:	Standard error mean
viz.	:	Videlicet (namely)

ABSTRACT

Tomato (Lycopersicon esculentum Mill.) is the third most important vegetable crop of India after potato and onion. It is affected by several fungal, bacterial, viral and nematode diseases. Among them post-harvest fruit rot of tomato is one of the major disease causing great loss to the vegetable. Tomato is cultivated both in kharif and rabi season. The most suitable temperature required for its cultivation is 15° to 27°C and can be grown in various kind of soils i.e. sandy loam to clay, black soil and red soil having suitable drainage condition. However, organic matter rich sandy loam soil is ideal for its cultivation. Due to its wider adaptability and versatility, tomato is grown all through the world Aspergillus fruit rot of tomato is one of the important fungal rot affecting the marketable fruit yield and production. Hence present investigations were conducted in Dept of Plant Pathology, OUAT, Bhubaneswar to study the post-harvest management of fruit rot of tomato caused by Aspergillus niger.

In present study a market survey was conducted in 6 different market places of Bhubaneswar region. Healthy and mature tomatoes along with rotted tomatoes were collected and taken to the laboratory in a clean polythelene for further studies. Artificial inoculation of Aspergillus was conducted which produced small, irregular black spots. These spots later enlarge to produce dark black spots and become watery. The reisolated culture from these artificially infected fruits by pinprick method proved to be same when compared with original culture.

All tested botanicals plant extracts were found effective in inhibition of mycelial growth of *Aspergillus niger*, Garlic clove extract (10%) and Coconut oil (2%) were found most effective in inhibiting the mycelial growth of *Aspergillus niger*. Maximum growth inhibition in *Aspergillus niger* was observed in treatment of Coconut oil and Garlic extract. All the tested chemicals were found fungistatic against *Aspergillus niger*. Calcium chloride and Boric acid at concentration 1%, were found most effective in inhibiting the mycelial growth of *Aspergillus niger*, while least inhibition of these fungi was observed with Potassium chloride and Sodium bicarbonate.

INTRODCTION

Tomato (Lycopersicon esculentum Mill.) is a one of the important vegetable crop belongs to Solanaceae family. It is perennial in growth habit, however usually cultivated as an annual. It ranks second after potato in world acreage and is the first processing crop amongst all. It is originated from Tropical America (Bose *et al*., 1986) however domesticated and cultivated first in Central America. Now it is widely cultivated throughout the year under green house condition. Tomato is cultivated both in kharif and rabi season. The most suitable temperature required for its cultivation is 15° to 27°C and can be grown in various kind of soils i.e. sandy loam to clay, black soil and red soil having suitable drainage condition. However, organic matter rich sandy loam soil is ideal for its cultivation. Due to its wider adaptability and versatility, tomato is grown all through the world.

Some of the major tomato producing countries are China, USA, Italy, India and Egypt. India ranks 2nd in terms of area and production after China. India is second largest producer of tomato next to China. In India, the area under tomato cultivation during the year 2017-18 was 801000 hectares with a total production of 22337 thousand metric tonnes having productivity 24.4MT per hectare. Some of the major growing states in India are Bihar, Karnataka, Odisha, Maharastra, Andhra Pradesh, Madhya Pradesh, Assam, Tamilnadu, Gujrat West Bengal etc. In Odisha condition Tomato is cultivated in both kharif and rabi season. Major tomato producing districts are Khurda, Kalahandi, Mayurbhanj, Keonjhar and Ganjam. However, it is cultivated as a rabi crop in all most all districts of Odisha, whereas kharif tomatoes are restricted to mid-hills at an elevation of 600-1000 mt above mean sea level.

The tomato is grown and eaten round the world for its nutritive and medicinal value. Tomato is rich in minerals, vitamins, natural acid, important amino acids and nutritional fibers. It is rich in vitamin A and C; it additionally incorporates minerals like P, S, Mg, Ca, Fe, K, Na, Cu and Cl. It can be used in various ways, such as raw in salads and processed into ketchup or tomato soup. Their intake is assumed to be good for heart amongst different matters because it bears accurate medicinal properties. They comprise lycopene, one of the most effective natural antioxidants and useful in

stopping prostate cancer. Tomato intake offers sturdy safety against neuro-degenerative diseases (Suganuma *et al.*, 2002).

These valuable crop plants going through loss because of many fungal, bacterial, viral and nematode diseases. Fruit rot is one of the important disease of tomato resulting in rotting of tomatoes with the aid of using microorganisms between harvesting and intake which ultimately make the fruits unfit for consumption. Different fruit rot diseases of Tomato are Alternaria rot caused by *Alternaria solani*, bacterial soft rot caused by Erwinia carotovora spp. carotovora (Jones) Holland, bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge), Anthracnose ripe rot caused by *Colletotrichum* spp; phoma rot incited by *Phoma destructive*, Cladosporium rot caused by *Cladosporium* spp; Fusarium rot caused by *Fusarium* spp., Helminthosporium rot caused by *Helminthosporium* spp, Rhizopus rot caused by *Rhizopus stolonifer* Lind., black mould rot caused by *Aspergillus niger*. Tomato is a completely perishable vegetable with brief shelf existence and because of their low pH, better moisture and nutrient composition cause them more prone to fungal diseases inflicting fruit rots. Improper harvesting, handling, packaging and transportation may also bring about bruises, decay and growth of microorganisms. Change in physiological state of fruit and storage circumstance make favorable condition for spoilage of fruit.

Fajola (1979) mentioned 25% loss at harvest and 3457 lack of the rem is product in transit, storage and marketplace because of post-harvest fruit rot of tomato in 5 states of Nigeria. Chinoko and Nagvi (1989) reported 243 fungi related to post-harvest rot of tomato from 8 advertising and marketing sites in Logos and Oyo states. Akthar *et al* (1994) reported that susceptibility of tomato to post harvest diseases induced to fungal pathogens throughout extended storage conditions. Prevalence of fungal fruit rot of tomato took place at some point of year inflicting extra or much less damage, however most losses took place throughout heat and humid condition (Chavan, 2012). The disease occurring in field and disease encountered after harvest are complementary to each other and need immediate action so that you can offer good enough and medical safety now no longer handiest for developing plant life withinside the field however additionally to plant produce after harvest throughout storage and transport.

OBJECTIVES

1) To detect the pathogen associated with the post-harvest rotting of tomato.
2) Their isolation, characterization & identification.
3) In vitro management of the pathogens with latest fungicides & botanicals.

REVIEW OF LITERATURE

A good numbers of research reports are available in literature on different aspects of the post-harvest fungal rots of tomato. However, literature pertinent to present investigation have been reviewed and presented under following sub headings.

2.1 Post harvest losses in tomato fruit due to fungal pathogen.
2.2 Types of Fungi Associated with Tomato Fruit Rot
2.3 Pathogenicity of associated fungi with tomato rots
2.4 Symptomatology of tomato fruit rots
2.5 Efficacy of chemicals against post-harvest fungal rot of tomato
2.6 Efficacy of Plant extract against post-harvest fungal rot of tomato
2.7 Post harvest management of tomato fruit rot
2.8 Impact of post-harvest treatment on marketable tomato fruits and spoilage

2.1 Post harvest losses in tomato fruit due to fungal pathogen.

Vandiar and Kamal (1966) described 63% losses of tomato because of rotting, while 10.65% losses during transport and storage because of *Alternaria alternata* had been described (Vir *et al.,* 1967). Losses as much as 40% is due to various post-harvest diseases at some point of the duration among harvest and consumption (Jamaluddin *et al.,* 1971).

A survey was conducted on the post-harvest fruit rot of tomato in five states of Nigeria and 25% loss was listed at harvest and 34% loss of the remaining product was during transport, storage and marketing by Fajola (1979).

Jaworski *et al.* (1977) published 25-50% losses of product due to *Phytopthora infestans* in USA.

The occurrence of fungal tomato fruit rot from vegetable markets and stores houses were 0.5-19.7% and 81.3% of the total festering because of *Phytophthora nicotianae* var. *parasitica, Fusarium equisetica, Fusarium palliodoroseum, Didymella*

lycopersici, Alternaria alternata and Geotrichum candidum was reported by Sharma (1994).

Mallek *et al*. (1995) published that the most common pathogens were *Alternaria alternata, Rhizopus stolonifer* and *Aspergillus niger* and post-harvest losses of tomato fruits to the mark of 52.7%, 35.9% and 25% respectively in Egypt.

Fontma *et al.* (1996) mentioned that no matter weekly sprays, crop losses due to *Alternaria solani* and *Phytopthora infestans* ranged from 12-67% and 14-52% during the early and late season respectively.

A survey was carried out on field for post-harvest sicknesses of hybrids and deshi cultivars of tomatoes in 4 districts of West Bengal and found out that amongst fungal sicknesses, *Alternaria* blight was the most predominant one having crop loss varies from 70-100% by Kanjilal (2000).

About 10-30% of total yield losses were due to the post-harvest diseases andin developing countries some perishable crops such as tomato, post-harvest produces were destroyed more than 30% of the crop yield was reported.

2.2 Types of fungi associated with tomato fruit rot

Thapa and Sharma (1975) mentioned that during storage of tomato fruit *Phoma destructiva, Colletotrichum phomoides, Phytophthora parasitica, Geotrichum candidum* and bacteria were the major causes of maximum damage in Solan areas.

Karla and Sohi (1985) during 1980 to 1984 conducted regular market surveys and reported that 78 fungal pathogens causing post-harvest diseases on 36 vegetable crops belongs to 23 genera and also described that among them *Alternaria* spp. and *Fusarium* spp. were the most persistent and devastating fungal pathogen.

Kulkarni (1985) observed that various genera viz., *Fusarium, Collectotrichum, Rhizopus, Alternaria, Phoma* and *Myrothecium* were responsible for post-harvest rots of tomato in Konkan region of Maharashtra. During 1983 a survey conducted at Madurai, Tamilnadu(India) revealed that association of 8 types of fungal pathogens in tomato rots viz., *Aspergillus niger, Rhizopus stolonifer, Fusarium semitectum,*

Oospora lactis, Rhizoctonia bataticola, Alternaria alternata and *Curvularia lunata* (Bhaskaran, 1986).

Chinoko and Nagvi (1989) isolated 243 fungi associated with post-harvest tomato rots from 8 marketing sites in Logos and Oyo states, among them *Aspergillus niger* van Tieghem and *Alternaria alternata* (Fries) Keissler were the most frequently isolated ones and mostly pathogenic.

7 fungi were isolated from rotten tomato fruits collected from 7 different markets of South-Western part of Nigeria, these includes *Aspergillus niger, Aspergillus flavus, Geotrichum candidum, Fusarium equiseti, Fusarium chlymodosporum, Acremonium recifei* and *Alternaria solani* by Oladiran and Iwu (1993).

Sharma (1994) observed the fungal rots in tomato fruits from vegetable markets and stores to the tune of 0.5 to 19.7 per cent. Amongst the fungi isolated, 81.3% of total spoilage was due to *Alternaria, Fusarium* and *Phytophthora* spp. Among the 11 fungal species causing rot on tomato fruits collected from different markets of Egypt, *Alternaria alternata* was recorded to the extent of 52.7% (Abdul Mallek *et al.*, 1995).

Ramgiry *et al*. (1997) reported that *Alternaria solani* and *Penicillium notatum* were the most frequent causal organism of decaying of tomato in fields and vegetable markets in Jhabua of Madhya Pradesh.

Choudhary (2002) reported that *Fusarium equiseti, Fusarium pallidoroseum, Collectotrichum gloeosporides, Cladosporium oxysporum, Geotrichum candidum, Phoma lycopersici, Alternaria alternata* and *Sclerotium rolfsii* were associated with tomato rot during storage and marketing in Himachal Pradesh.

Sharma and Chaudhary (2004) reported 8 genera *Fusarium, Collectotrichum, Cladosporium, Geotrichum, Phoma, Alternaria, Rhizopus, Didymella* and *Sclerotium* during the survey of various districts of Himachal Pradesh with tomato rots.

Muhammad *et al*. (2004) reported that 8 fungi viz., *Aspergillus niger, Aspergillus ochraceous, Aspergillus flavus, Aspergillus fumigates, Penicillium citrinum, Helminthosporium flavum, Curvularia lunata* and *Sclerotium rolfsii* were associated with tomato rots during survey of different markets of Sokoto town of North-Western Nigeria in 2001 and 2002.

Kutama *et al*. (2007) conducted a survey in Rimi market, Kanometropolis of Nigeria region and isolated 200 fungal isolates associated with tomato rots and classified them into 5 genera namely *Aspergillus* spp., *Alternaria* spp., *Rhizophus* spp., *Penicillium* spp. *and Saccharomyces* spp.

Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer and *Geotrichum candidium* were isolated from the periphery of a rotten tomato fruits by tissue isolation method on Potato Dextrose Agar medium by Yeni *et al*. (2010)

Chavan (2012) isolated 9 fungi from rotten tomato fruits collected from different markets of Konkan region of Maharashtra, these includes *Alternaria solani, Alternaria alternata, Alternaria citri, Aspergillus niger, Collectotrichumg loeosporides, Fusarium oxysporum, Syncephalastrum racemosum* and *Cunnighhamella elegans*.

Amuji *et al*. (2013) isolated organisms from the tomato samples associated with soft rot in tomato fruits. They observed that *Fusarium oxysporum* and *Rhizopus stolonifer* were the predominant fungal pathogens isolated from tomato fruits and considered as the most endemic fungal pathogens affecting tomato in Nsukka South-Eastern Nigeria.

Lemma *et al*. (2014) isolated four fungal pathogens i.e. *Rhizopus* spp., *Alternaria* spp., *Fusarium* spp. and *Penicillium* spp. from the infected tomato fruit samples, both in the on and off cropping season and observed maximum percentage frequency of occurrence of *Rhizopus* spp. (25%), *Alternaria* spp. (16.7 and 8.3 per cent), *Fusarium* spp. (16.7 and 33.3 per cent) and *Penicillium* spp. (41.6 and 33.4 per cent).

Sajad *et al.* (2017) conducted three year survey during 2013 -16 for prevalence of post-harvest diseases of tomato in local markets of Jabalpur district of Madhya

Pradesh and found that most of the tomato fruits were suffered by fruit rot diseases caused by *Alternaria alternata, Aspergillus niger, Geotrichum candidum, Alternaria solani, Mucor racemosus, Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme, Penicillium digitatum, Rhizoopus stolonifer, Colletotrichum lycopersici, Sclerotium rolfsii, Myrothecium roridum, Phoma destructiva and Trichothecium roseum.* They observed that amongst the associated fungi with tomato rot, *Alternaria alternata* (16.51%) had highest frequency of occurrence followed by *Alternaria solani* (12.43%), *Geotrichum candidum* (10.66%), *Aspergillus niger* (8.82%) and *Colletotrichum lycopersici* (7.53%).

2.3 Pathogenicity of associated fungi with tomato rots

Injury to fruits was found effective in initiating different kinds of rots in fruits (Granger and Horne, 1924).

Thakur and Chenulu (1970) observed the decay of tomatoes from Delhi. The diseases were *Alternaria* rot (*Alternaria tenuis*); soft rot (*Rhizopus stolonifer* Ehrenberg); *Fusarium* rot (*Fusarium* spp.) and black mould rot (*Aspergillus niger*) further they had also reported the detailed symptoms produced by each pathogen causing aforesaid diseases.

Mehta *et al.* (1975) proved the pathogenicity of *Alternaria solani* and *Alternaria tenuis* causing fruit rot of tomato by injuring tomato fruits. While Khanna and Chandra (1976) established the pathogenicity of fungal pathogens by inoculating the healthy fruits by employing pinprick method.

Hasija and Batra (1979) performed pathogenicity test on raw and ripe tomato fruits. Their findings indicated that the *Phoma destructiva* pathogen was a wound parasite and observed that more disease severity in ripe than in raw fruits.

Kulkarni (1985) made number of isolation of fungi from diseased tissue of fruits on potato dextrose agar medium and established pathogenicity by making injuries with sterilized sand paper at shoulder region of fruits.

There are also reports of *Aspergillus niger* induced spoilage of tomatoes (Sinha and Saxena, 1987).

Yen Hui *et al*. (1994) studied the biology and pathogenicity of *A. solani* on tomato and revealed that wounds as a necessary for successful invasion of pathogen.

Kwon *et al*. (2008) observed on the basis of pathogenicity test that infection of *Penicillium oxalicum* on tomato occurred through wounds and cracks on the tomato fruits.

Taskeen-un-Nisa *et al.* (2011) also revealed that infection of various fungi on tomato occurred through wounds and cracks on the tomato fruits.

Oladiran and Lwu (2011) reported that seven fungi associated with fruit rot of tomato were isolated including *Fusarium equiseti, Fusarium chalmydosporum, Alternaria solani, Geotrichum candidium, Acremonium recifei, Aspergillus flavus* and *Aspergillus niger*. They were all pathogenic on tomato fruits, most pathogenic being *Geotrichum candidium* followed by *Aspergillus niger*. Least rot was caused by *Alternaria solani.*

Chaurasia *et al*. (2013) proved the pathogenicity of *A. solani* on green, semi ripe and mature tomato fruit by making a small injury on the surface sterilized tomato fruit followed by inoculation of agar disc taken from margin of the freshly grown colony of isolated pathogen.

Lemma *et al*. (2014) carried out pathogenicity test of *Rhizopus* spp., *Alternaria* spp., *Fusarium* spp., *Penicillium* spp. and revealed that none of these pathogens initiate the rot symptoms when inoculation was made on unwounded tomato fruits.

Onuorah and Orji (2015) conducted pathogenicity test and showed that *Aspergillus niger* had the highest decay diameter of 30 mm in the healthy tomato fruits while *Geotrichum candidum* had the lowest decay diameter.

Onuorah and Orji (2015) established the pathogenicity of isolated fungi associated with tomato rot on surface sterilized tomato by making bore holes with sterile cork borer followed by inoculation of isolated fungi and sealing of holes with sterile petroleumjelly.

2.4 Symptomatology of tomato fruit rots

Samuel (1932) reported that *A. solani* was mainly responsible for the tomato decay and produced typical symptoms of brown to black depressed spot and usually with distinct rings.

Illman *et al*. (1959) studied the symptoms of anthracnose of tomato rot on young fruit and they observed that symptoms were in the form of circular lesion with later became dark, sunken and partially covered with salmon pink spore mass often in concentric circles.

Bartz (1971) noticed *Alternaria* rot causing lesions as firm, flattened or slightly sunken, with or without a definite margin and often with dense velvety olive green or black spore masses on the surface of fruit.

Datar and Mayee (1981) observed that pathogen *Alternaria solani* produced small dark brownish to black leathery spot with faint concentric margins. These spots later enlarged and covered the completely upper portion.

Stevenson (1991) reported that infected fruit have a short shelf and disease results in serious fruit loss if not controlled. Some authors also reported *Colletotrichum coccodes* and *Colletotrichum gloeosporioides* of tomato.

Sinha and Saxena (1997) observed the soft rot developed with *Aspergillus niger*. The affected tomato fruits showed characteristic blackgrowth of fungi with secretion of liquid and emittence of odour and finally whole fruit spoiled within 3-4 days of infection.

Muhammad *et al.* (2004) observed that many fungi produced types of black rots on number of vegetables and fruit crops.

Markson *et al*. (2005) who observed that *Rhizopus stolonifer* and *Fusarium* spp. were responsible for tomato soft rot.

2.5 Efficacy of chemicals against post-harvest fungal rot of tomato

Ivanovic *et al*. (2002) evaluated the inhibitory effects of sodium bicarbonate (SBC) on conidial germination and mycelial growth of *Alternaria solani* at various

concentration of 0.5%, 1.0%, 2.0% and 5% and result revealed that conidial germination was inhibited to the extent of 72-95%, whereas inhibitory effect of SBC on hyphal growth was not statistically significant.

Nene and Thapliyal (2002) suggested 0.2 per cent Maneb or Mancozeb found very effective against *Aspergillus niger, Alternaria alternata* and *Penicillium digitatum* which caused post-harvest fruit rot of tomato. They recommended spraying of 1000 ppm Caftafol or Benziinidazoles for control fruit rot of mango caused by *Aspergillus niger.*

Patel *et al.* (2005) evaluated chemicals viz., copper oxychloride, mancozeb, carbendazim, boric acid and potassium permagnate *in-vitro* for their effect on growth and spore germination of *Alternaria lunata* inciting fruit rot of tomato. Copper oxychloride and mancozeb were found more effective in inhibiting the growth and spore germination of *Alternaria lunata*.

Abdel Mallek *et al.* (2011) evaluated that fruit rot caused by *Alternaria alternata* in tomato can be effectively controlled by Benlate, Rovra and Sumiclex while rotting caused by *Alternaria* and *Aspergillus niger* effectively controlled by Sodium hypochloride while Cuprosan and Ridomil were ineffective against rotting caused by *Alternaria alternata* and *Aspergillus niger.*

2.6 Efficacy of plant extract against postharvest fungal rot of tomato

Sumba G and Mehrotra (1980) reported that garlic extract was effective in controlling *Aspergillus niger, Gliocladium roseum* and *Sclerotium rolfrii* causing peach, apple and pear fruit rot, respectively. Dey and Choudhary (1984) studied the antimicrobial activity of *Ocimum sanctum* L. oil (5%) against *Alternaria solani* and showed the considerable activity against fungus. The plant extract of *Allium sativum* (Garlic) at 10% and *Vinca rosea* (Sadafuli) at 10 per cent inhibited completely the mycelial growth and spore germination of *Alternaria solani*. The inhibitory effect of garlic bulb extract (10%) on mycelial growth of *Alternaria tenuis* causing brinjal leaf spot was reported (Datar, 1992).

Montes and Garcia (1997) studied the effect of 50 plant extracts on spore germination of *A. solani,* of which extract of *Allium sativum* L., *Teloxysam brosioides*

and *Tagetus erecta* showed strong inhibitory actions, four extracts increased the spore germination and the rest were undifferentiated.

Prasad and Naik (2003) evaluated five plants extracts with different concentration at 2.5, 5.0 and 7.5 per cent against *Alternaria solani*. Among all plant extracts tested, garlic extract was the most effective one with 90.7 per cent inhibition of mycelial growth followed by Prosopis leaf extract (79.9%) at a concentration of 7.5%. Ocimum leaf extract and neem leaf extracts were the next best with 65.6 % capacity to inhibit mycelial growth.

Varma and Gandhi (2006) evaluated some phytoextracts against *Alternaria solani*, the causal agent of early blight of tomato, under laboratory and net house conditions. Among the phyto-extracts tested in-vitro, cold aqueous extracts of *Clerodendron aculeatum* and *Azadiracta indica* showed maximum inhibition of mycelial growth of *Alternaria solani* and foliar spray of Clerodendron leaf extract (15° o) immediately after appearance of symptoms with the test fungus was found effective in reducing the disease severity under screen house conditions.

Irkin and Korukluoglu (2007) reported that onion extract with ethyl alcohol (275 mg/mL MFC), aqueous garlic extract (325 mg/mL MFC) and aqueous leek extract (900mg/mL MFC) found the most inhibitory against *A. niger*.

Babu *et al.* (2008) reported that extraction of plant parts with ethanol and acetone were more inhibitory than water extractions to fruit rot pathogen *Colletotrichum gloeosporioides*. Extracts of *Azadirachta indica, Tagetes erecta* and *Annona squamosa* were effective in inhibiting the growth of the fungus.

Jadhav *et al.* (2008) reported that garlic clove (10%) extract was effective in inhibiting the growth of *Colletotrichum gloeosporioides.*

Mundhe (2009) reported that leaf extracts of *Azardirachta indica* (20% and 10%) bulb extracts of *Allium sativum* were highly effective in controlling the growth of *Alternaria solani.*

Gachande (2010) extracts of 15 plant parts were tested against spore germination and mycelial growth of *Alternaria solani* isolates. The extracts of *Allium sativum* was found to be most effective in regulating the growth of fungus.

Sheth *et al.* (2010) and Mohamed *et al.* (2012) observed that garlic cloves, onion and neem leaf extracts had the ability to cause reduction in the mycelial growth of *A. niger*.

Vishwapal *et al.* (2013) reported that extract of neem leaf most effective in inhibiting mycelium growth as well as in preventing of spore formation of *A. niger*.

Hegde *et al*. (2014) evaluated nine different plant extracts against *Colletotrichum gloeosporioides, viz., glyricidia*, neem, onion, eucalyptus, duranta, ocimum, garlic, ginger at two concentrations (5 and 10 per cent) and Azadirachtin (2.5 and 5.0 per cent). Azadirachtin was found to be the best in mycelial growth inhibition (89.25%) followed by ginger (48.05%) and onion (38.05%) whereas Azadirachtin and garlic clove extract were best in complete inhibition of sporulation of *C. gloeosporioides.*

Ethanolic extracts of tarragon, rosemary, thyme, and the essential oil of oregano are powerful inhibitors of *Penicillium* spp., *Rhizopus* spp., *Fusarium* spp., *Aspergillus niger* and *Aspergillus flavus* (Ibrahim and Al-Ebady, 2014).

Roopa *et al.* (2014) reported that Jatropa leaf extract @ 10 per cent was found effective in inhibiting the mycelial growth of *A. solani* (62.78%).

2.7 Post harvest management of tomato fruit rot

Vir *et al*. (1967) reported that aureofungin as post-harvest dip treatment prolonged the life of tomato fruits by 11-12 days whereas rotting occurred in untreated fruits within 2-3 days.

Bengerth *et al.* (1972) reported that post-harvest treatment with calcium was found effective in extending the storage life of many fruits by maintaining their firmness and minimizing the rate of respiration, protein break and rotting incidence.

Sokhi and Sohi (1972) reported the effectiveness of fruit dip treatment with cuman L (0.1%) and dithane M-45 (0.05%) against *Fusarium* and *Alternaria* rots of tomato.

Tanaka and Nonaka (1981) found that post-harvest bulb rot in onion was reduced 16 to 17 per cent when dusting of topped onions with calcium carbonate was carried out.

Pre and postharvest calcium applications had been used to delay ageing or ripening to reduce post-harvest decay and control of many diseases in fruits and vegetables (Poovaiah. 1986).

Dang and Gupta (1984) studied the chemical control of storage rot of onion bulbs caused by *Fusarium solani* and found that pre inoculation treatment with 2,4-D. potassium meta-bisulphite diphenylamine and acetic acid gave 5S per cent reduction in rotting of onion bulbs.

Bhaskaran and Narasiman (1988) proved effectiveness of fruit dip treatments with various chemicals when used as pre inoculation treatment rather than post inoculation treatment. Among the 19 products Cumin L., Bavistin and Dithane M-45 significantly recorded the maximum inhibition of *Rhizopus* rot development. While Neem oil followed by Fuji-1. Castor oil and Curran L. recorded the maximum inhibition of *Fusarium* rot.

Prasad (2002) tested nine post fungicides including botanicals. bioagents and wax coating against *Alternaria* fruit rot of tomato both by pre-inoculation and post inoculation treatment and observed that among the fungicide tested iprodione (0.2%) was found effective in reducing the post-harvest decay of tomato both at pre-inoculation and post inoculation treatment followed by wax coating and mancozeb.

Bombelli and Wright (2006) evaluated the effect of potassium bicarbonate (KHCO3) on tomato fruit quality during storage and observed that KHCO3 @ 2% concentration fruit dip treatment was sufficient to prevent the attack of *B. cinerea* for two weeks and maintaining the fruit quality. Treatmentwith 0.5 or 1% KHCO3 was

sufficient to protect fruit against B. cinerea, whereas treatment with 3% KHCO3 for 4 min decreased fruit quality.

Meena *et al*. (2006) evaluated fungicides and plant extracts for control of *Alternaria* fruit rot of brinjal and found that all fungicides and plant extracts were significantly superior over control on 4th and 7th day of pre and post inoculation treatment. Bavistin (0.1%) among fungicide gave lowest disease severity followed by raxi and inofil, whereas among plant extracts *Ocimum sanctum* leaves extract gave lowest disease incidence. A good control of *Fusarium* fruit rot of tomato was achieved by using *Azadirachta indica*, *Allium sativum*, *Ocimum sanctum* and *Curcuma longa* (Singh and Jain, 2007).

Asrin *et al.* (2008) studied the effect of chlorine, packaging and storage condition on quality and shelf life of tomato and observed that the shelf life of tomato was extended up to 17 days without excessive deterioration in quality by treating the fruits with chlorine, packed in perforated polyethylene bags and kept at ambient temperature as compared to control for 7 days only.

Patel *et al*. (2008) reported that the extract from *Allium sativum* was significantly better followed by extract of *Jatropha curcas*, *Aloe barbadensis* and *Azadiracta indica* in controlling *Aspergillus niger* of aonla fruit rot.

Un-Nisa *et al.* (2010) studied the efficacy of extracts obtained from mint leaves, onion bulbs and garlic on the spore germination of *Rhizopus stolonifer* and *Alternaria alternata*. Higher conc. found highly effective followed by lower conc. The results further indicated that the extract of *Allium sativum* was highly effective as compared to *Allium cepa* and *Mentha arvensis.*

Wang *et al*. (2010) showed that the combine treatments of *Rhodosporidium paludigenum* 1 x 108 cells ml^{-1} and $CaC1_2$ at the concentration from 0.5 to 2% were effective in reducing the black rot caused by *Alternaria alternata* in cherry tomato wounds than those of *R. paludigenum* or $CaCl_2$ alone. Meanwhile, 0.5% $CaCl_2$ in combination with 1 x 108 cells ml^{-1} *R. paludigenum* greatly inhibited the natural decay of cherry tomatoes in 21 days' storage at 25°C.

Chavan and Tawade (2012) reported that among plant extracts, garlic clove extracts @ 10% was found most effective and recorded least fruit rot severity caused by *A. solani* on 4th day and 7th day followed by Nilgiri oil and also observed that biosafe @ 1.0% was most effective chemical followed by calcium chloride @ 4.0% on 4th day and 7th day in reducing the fruit rot intensity.

Tasiwal *et al.* (2012) reported that under in-vivo evaluation of salts, biocontrol agents and hot water treatment at 49°C for 20 minutes, the least PDI was found in case of sodium chloride + hot water treatment (26.67%) followed by sodium chloride (33.33%), hot water treatment (46.67%) and *T. harzianum* + *Pseudomonas fluorescence* (43.33%) at 5 per cent concentration and more growth of fungus was noticed in *T. harzianum* (76.67%).

Khiareddine *et al.* (2016) screened potassium sorbate, potassium biocarbonate and dipotassium hydrogen phosphate for their antifungal activity against *Fusarium oxysporum* f. spp. *lycopersici, Fusarium oxysporum* f. spp. *radicis, Fusarium solani, Verticillitim dahlia, Rhizoctonia solani, Colletotrichum coccodes, Pythium aphanidermatum, Sclerotinia sclerotiorum, Botrytis cinera* and *Alternaria solani* and observed that DPHP and PS fruit treatment had recorded significantly decreased *Botrytis cinera, Rhizoctonia, Alternaria* and anthracnose tomato fruit rots by 46.68% and 30.81%, 14.4% and 15.74%, 20.00% and 31.67% and 19.17% and 25.24% respectively compared to inoculated and untreated control.

Damaram (2013) reported that *Fusarium* fruit rot severity in tomato fruits treated with neem leaf extracts both in pre and post-inoculation treatments showed lower fruit rot severity (18.33 & 19.33 %) as compared to control (47.33 %) after 8 days of inoculation.

Sandipan *et al.* (2014) tested leaf extracts of five plant species datura (*Datura stramonium*), neem (*Azadirachta indica*), garlic (*Allium sativum*), nilgiri (*Eucalyptus citridora*) and lantana (*Lantana camera*) against fruit rot of tomato caused by *Alternaria* and observed that both in pre and post inoculation treatments, datura extract (100%) was found most effective followed by leaf extracts of neem, garlic and nilgiri, while unsterilized leaf extract of lantana was found to be least inhibitory. Pre-treatment was found to be more effective than the post-treatment.

Pandya (2015) observed that treatment of aluminium acetate and aluminium chloride were effective in reducing the *Fusarium* dry rot of potato tubers treated on 8th day after inoculation in pre and post-inoculation treatments.

2.8 Impact of post harvest treatment on marketable tomato fruits and spoilage

Prasad and Naik (2003) reported that iprodione, mancozeb, waxol and prosopis leaf extract treated fruits in pusa ruby cultivar had lower per cent loss in weight and acidity and increased TSS.

Asrin *et al*. (2008) observed the effectiveness of treatment of tomato fruits with chlorine followed by packing in perforated (0.25%) polyethylene bags and storing at room temperature on quality of tomato fruits. They observed a substantial reduction in losses caused by decaying and weight loss. This combine treatment also considerably delayed compositional changes in TSS, total sugar, reducing sugar, vitamin-C, B-carotene etc. They also observed that shelf life of tomato had extended up to 17 days as compared to untreated ones.

Senevirathna *et al*. (2010) treated mature tomato fruits (CV. 'Thilina') with four different concentrations of $CaCl_2$ (0%. 2%. 4% and 6% aqueous solutions) using different modes of application: dipping, vacuum infiltration and pressure infiltration to improve the shelf life and quality and observed that vacuum infiltration was the most effective one with respect to shelf-life extension and 6% $Cacl_2$ treatment at 20 kPa was also the best in terms of extension of shelf life (92%) and in maintaining the post -harvest qualities compared to untreated fruits kept at 28°C.

Genanew (2013) tested different concentrations of $CaCl_2$ with and without modified atmosphere on storage behaviour and quality of tomato (Lycopersicon esculentum, Mill.) fruits under laboratory conditions to test the effect of post harvest treatments to prolong shelf life of perishable fruits and observed that $CaCl_2$ had delayed the ripening rates by facilitating the retention of firmness, quality and improving shelf life.

MATERIAL AND METHODS

Present research work entitled " Microbial Association with harvested tomato fruits & its characterization." was conducted at the Department of Plant Pathology college of Agriculture, OUAT, Bhubaneswar during the year 2020-21. The details about materials used and methods adopted during the course of research work are described below in this chapter**.**

3.1. General Laboratory Procedure

Throughout the research work, Borosil make Petri plates, conical flasks and test tubes were used. The cleaned dried glass wares were sterilized in hot air oven at 160°C for two hour. The media and distilled water were sterilized in autoclave at 151b pressure for 15 minutes.

3.1.1 Materials

The materials required to conduct the experiments included chemicals for culture media, botanical or plant extracts, glass wares, equipments and few miscellaneous articles.

3.1.1.2 Chemicals

(a) Cleaning solution

Cleaning solution contained potassium dichromate 80g, distilled water 300 ml and concentrated sulphuric acid 400 ml. This was used to clean the glass wares.

(b) Mercuric chloride solution

1:1000 solution of HgC12 was prepared and used for surface sterilization of diseased samples.

(c) Mounting medium

Lacto phenol and cotton blue with the following composition were used as the staining media for studying the characteristics of the pathogen.

(d) Lacto phenol

Phenol (pure crystals, liquefied to 20 ml by gentle heating on water bath)

Lactic acid	20 ml
Glycerol	40 ml
Distilled water	20 ml

(e) Cotton blue

Anhydrous lacto phenol	67 ml
Distilled water	20 ml
Cotton blue	0.1gm

3.1.1.3. Media:

Ingredients used for the preparation of the culture media during the study have been given below: Potato dextrose agar medium

Potato dextrose agar (PDA) medium having the following ingredients was used for a few physiological studies.

Peeled and Sliced potato (extract) 200 g

Dextrose	20 g
Agar -agar	20 g
Distilled water	1 Lit.
pH (adjusted to)	6.5

3.2 Identification of fungi associated with tomato rots.

3.2.1 Collection:

Diseased (rotten) tomato fruits were collected from different local markets such as Siripur, Ganganagar, Soubhagya Nagar, Delta square, CRP square and Unit-1 market of Khurda district in the month of January. Randomly selected mature fruits along with the diseased ones were brought to the laboratory of department of Plant Pathology in clean polythene bags for further study.

Table 1. List of markets from which samples were collected

Sr. No.	Market Name
1.	Siripur
2.	Ganganagar
3.	Soubhagya nagar
4.	Delta square
5.	CRP square
6.	Unit -1 market

3.2.2 Visual observation:

Visual observations were recorded to find out manifestation of different symptoms of fruit rot of tomato.

3.2.3 Microscopic examination:

Diseased fruits were washed in tap water to remove extraneous material. Slides were prepared by cutting fine sections from the diseased specimens in lactophenol and observed under the compound microscope.

3.3 Isolation of organism:

Number of isolations were prepared from diseased tomato fruits separately on potato dextrose agar (PDA) medium by usual isolation method. The infected samples collected were cleaned thoroughly with sterile water to remove extraneous material. Then air dried and small pieces of infected portion along with healthy portion were taken. These pieces were surface sterilized with 0.1% mercuric chloride for 1 minute and then washed several times with sterile water to remove the traces of the disinfectant if any. The pieces were flame dried and were plated under aseptic condition on agar medium previously sterilized at 15 lb pressure for 15 minutes. The Petri plates were incubated at room temperature (27± 2°c) until proper growth of fungi was obtained. Growths of the fungi were obtained within 3 to 5 days in all Petri plates. Bits of small mycelial growth from the typical colonies were transferred on slants of PDA under aseptic condition. The isolates were maintained separately in pure state on PDA slants for further studies.

3.4 Pathogenecity of isolated fungi

3.4.1 Inoculation:

For the inoculation process mature healthy tomtoes of uniform size and colour were taken. They were surface sterilized with 0.1% mercuric chloride for 1 minute and rinsed thoroughly with sterile water. Five set of polythelene packets were taken. In each packet a set of 2 tomatoes were taken. Two packets are taken as control purpose. From the remaining two one packet containing fruits were pin pricked to a depth of 2-3 mm and other was left as such. Two sets are inoculated with spores of Aspergillus. Inside each packet cotton swabs soaked in water were kept to maintain the humidity. Four to five holes were made on the polythene for maintaining humidity and kept under the room temperature. After 72 hours of inoculation the polythelene were opened and fruits were observed for symptom developments. In the control packet the tomatoes remain as such. Symptoms were seen to be developed on the inoculated ones.

3.4.2 Reisolation:

Reisolation was done from the artificially inoculated fruits. The fungal cultures obtained on PDA by reisolation were compared with the original culture obtained from naturally infected fruits.

3.5 Identification of the causal organism:

The cultures were tentatively identified using cultural and morphological features.

3.6 Study of symptoms of tomato rots produced by Aspergillus:

The developed symptoms were recorded on artificially inoculated fruits which were continued till the development of typical symptom achieved due to each isolate.

3.7 In-vitro efficacy of plant extracts

An experiment was carried out to evaluate the possible fungitoxic effects of

different plant extracts viz., ginger, garlic, mustard oil, coconut oil and til oil against Aspergillus fruit rot of tomato. The effect was tested through Poisoned Food Technique. The plant extracts were prepared by adopting aqueous plant extract solution. The standard aqueous extracts of plant materials were obtained by grinding the appropriate washed plant materials in mortar and pestle in presence of equal amount of sterile distilled water. The plant extracts were then filtered with the help of muslin cloth. The plant extracts along with their used concentration are given in Table. Potato dextrose agar medium was prepared and poured in 250 ml conical flask and autoclaved at 15 lb pressure for 15 minutes. Before solidification of media different plant extracts with desired concentration were incorporated aseptically in flasks. These flasks were shaken thoroughly and poured in Petri plates at the rate of 20 ml /plate. One set of plate was poured without plant extracts to serve as control. The inoculated plates were kept in the inverted position. Plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogen on medium were recorded and per cent inhibition over control was calculated by the following formula of [Horsfall, 1956.]

$$X = \frac{[Y - Z]}{Y} \times 100$$

Where, X = Per cent inhibition.

Y = Growth of fungus in control (mm)

Z = Growth of fungus in treatment(mm)

Table 2. List of plant extracts used against Aspergillus fruit rot in tomato

Sr. No.	Botanical name	Common name	Family	Plant Part Used	Conc. (%)
1.	*Allium sativum*	Garlic	*Liliaceae*	Clove	10%
2.	*Zingiber officinale*	Ginger	*Zingiberaceae*	Rhizome	10%
3.	*Brassica nigra*	Mustard	*Brassicaceae*	Oil	2%
4.	*Cocus nucifera*	Coconut	*Arecaceae*	Oil	2%
5.	*Sesamum indicum*	Sesame	*Pedaliaceae*	Oil	2%

3.8 In-vitro efficacy of chemicals against Aspergillus fruit rot

The effect of boric acid, potassium chloride, calcium chloride, sodium chloride were tested in-vitro separately at 0.5% and 1% concentrations respectively and sodium bicarbonate at 1% and 2% concentration on mycelial growth of Aspergillus fruit rot of tomato (Table 3). Each chemical was mixed to PDA medium at a required concentration. Petri plates containing PDA without chemicals was served as control. After medium solidification plates were inoculated with 5 mm discs of 7-day-old culture of each test fungus and incubated at 25 ± 2°C for 7 days. The colony diameters of the fungal pathogens on medium were recorded and per cent inhibition over control was calculated by the following formula suggested by Horsfall (1956).

$$X = \frac{[Y - Z]}{Y} \times 100$$

Where, X = Per cent inhibition.
Y = Growth of fungus in control (mm)
Z = Growth of fungus in treatment(mm)

Table 3. List of chemicals used against Aspergillus fruit rot of tomato

Sr. No.	Name of chemicals	Conc. (%)
1.	Boric acid	1 %
2.	Calcium chloride	1 %
3.	Potassium chloride	1 %
4.	Sodium chloride	1 %
5.	Control	

3.10 Effect of post infection treatment of botanicals, chemicals and fungicides on intensity of fruit rot of tomato.

Fruit dip treatments of effective botanicals. chemicals and fungicides found effective in in-vitro study against pathognic isolate of Aspergillus niger causing fruit rot of tomato were followed by post infection method. Healthy and mature tomatoes of uniform size were used for the experiment. In post inoculation treatment healthy tomato fruits were first surface sterilized with 0.1% mercuric chloride and then washed thoroughly with sterilized water. The fruits were first dipped in different botanicals, chemicals and fungicides for 10 minutes and kept for 12 hours at room temperature with 100% relative humidity. Test fruits were pin pricked to a depth of

nearly 2-3 mm and inoculated with the culture suspension having mycelium and spores of fungus from 8 days old culture. Three set of 3 fruits were maintained for each treatment with control in post infection methods. Observation on disease intensity on fruits was recorded by 0-5 scale (Table 4) on 8th day after treatment.

Table 4. Standard disease score chart for accessing PDI of fruit rot of tomato (0-5 scale)

Rating Scale	Percent disease on fruit surface
0	No disease
1	0.1 - 5
2	5.1 - 10
3	10.1 - 25
4	25.1 - 50
5	More than 50

Per cent disease intensity (PDI) was calculated by using the formula proposed by Wheeler in 1969

$$PDI = \frac{\text{Total numerical ratings}}{\text{No of samples x Maximum disease grade}} \times 100$$

3.11.1 Spoilage

Fruit was considered spoiled when it showed dehydration, rotting or other abnormal symptoms on more than 10% of its surface.

3.15 Statistical analysis

The data obtained through all the experiments were statistical 20 analyzed (Parse and Sukhatme, 1985). The per cent values were transformed into arcsine values. The standard error (SE) and critical difference (C.D.) at level P= 0.5 and 0.1 were worked out results obtained were compared statistically. All the statistical analysis was done using OP-STAT statistical programme at Central Computer Laboratory, OUAT, Bhubaneswar.

RESULTS

A survey was conducted in six different markets of Bhubaneswar region namely Siripur, Ganganagar, Soubhagya nagar, Delta square, CRP square and Unit-1 market. The infection percentage varied from 1-5%. Maximum percentage was observed in siripur and unit-1 market.

Table 5. Calculation of disease percentage

Sr. No.	Market Name	Disease percentage
1	Siripur	5%
2	Ganganagar	2%
3	Soubhagya nagar	2%
4	Delta square	1%
5	Crp square	2%
6	Unit-1 market	5%
	Mean	2.8%

4.1 Collection & Isolation of *Aspergillus niger*

Typical fruit rot infected tomato samples were collected from the market. Standard tissue isolation technique was followed to obtain *Aspergillus niger* from the infected samples showing typical fruit rot symptoms. Isolations were repeated several times to obtain the pure culture. The pure culture thus obtained was identified as *Aspergillus niger* on the basis of morphological characters. The cultural and morphological characters were found similar to *Aspergillus niger* causing fruit rot of tomato.

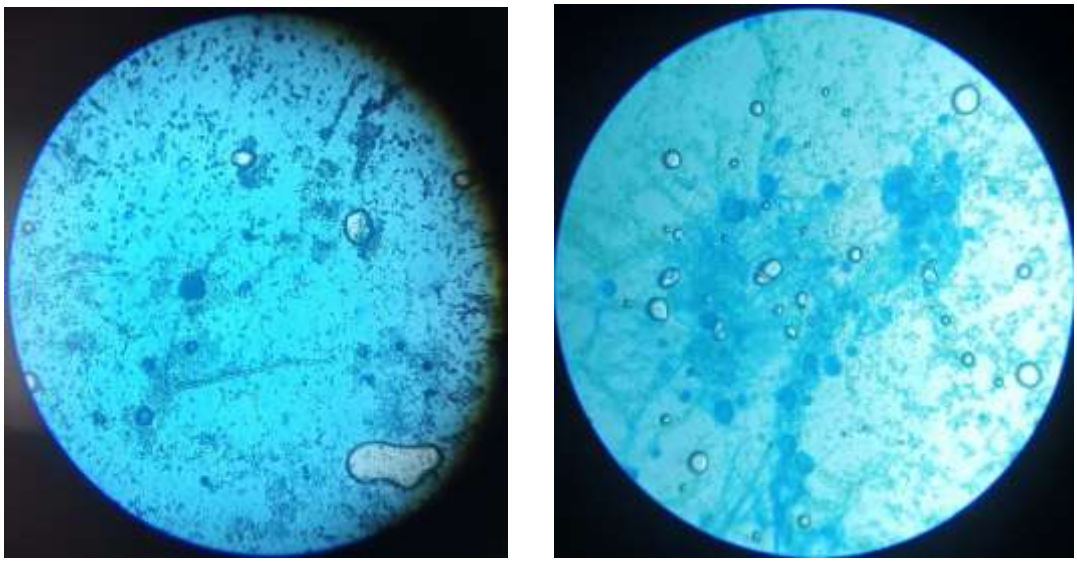
4.3 Pathogenecity Test of *Aspergillus niger*

The isolates produced fruit rot symptoms within 3-5 days of inoculation in the form of small, irregular black spots. These spots later enlarge to produce dark black spots and become watery. The reisolated culture from these artificially infected fruits by pinprick method proved to be same when compared with original culture.

Fig 1

Pure culture of *Aspergillus niger*

Fig 2



Micro scopic images of *Aspergillus niger* (condia and conidial head)

Fig 3

T1

T2

T3

Control

Pathogenecity of post harvest tomato fruit rot and their symptoms

Similarly, previous workers established the pathogenicity of tomato by injuring the fruit or by pinprick method.

4.4 Study of symptoms of fruit rot of tomato caused by *Aspergillus niger*

Aspergillus produces filamentous hyphae which make them look like small plants. Aspergillus produces water-soaked lesions on developing which became puffy and exude liquid and infection sites showed grayish white growth which turned black at later stages and finally fruits become watery and completely rotted.

4.5 In-vitro efficacy of plant extracts against *Aspergillus niger*

Efficacy of five plant extracts were tested using Poisoned Food Technique (PFT) for inhibition of mycelial growth of *Aspergillus niger*. Evaluation of different plant extracts for their fungitoxic properties against *Aspergillus niger* showed significant inhibition of growth of test fungi in-vitro over control. Data revealed that radial mycelial growth of *Aspergillus niger* was recorded against 88.23 mm in untreated control. However, significantly least mycelial growth was recorded with maximum inhibition of 98.99% with treatment of Coconut oil which was at par with treatment of garlic clove extract (98.89%) followed by ginger rhizome extract (72.88%), mustard oil (72.01%) and sesamum oil (61.34%).

Table 6. In vitro efficacy of plant extracts against *Aspergillus niger*

Tr. No.	Treatment	Colony Dia. * (mm)	Per cent Inhibition
T_1	Ginger (Zingiber officinale)	24.54	72.88
T_2	Garlic (Allium sativum L.)	1.25	98.89
T_3	Mustard oil (Brassica nigra)	25.00	72.01
T_4	Coconut oil (Cocos nusifera)	1.02	98.99
T_5	Sesamum oil (Sesamum indicum)	34.56	61.34
T_6	Control	88.23	00.00
	SE± CD @ 1%	00.62 02.85	

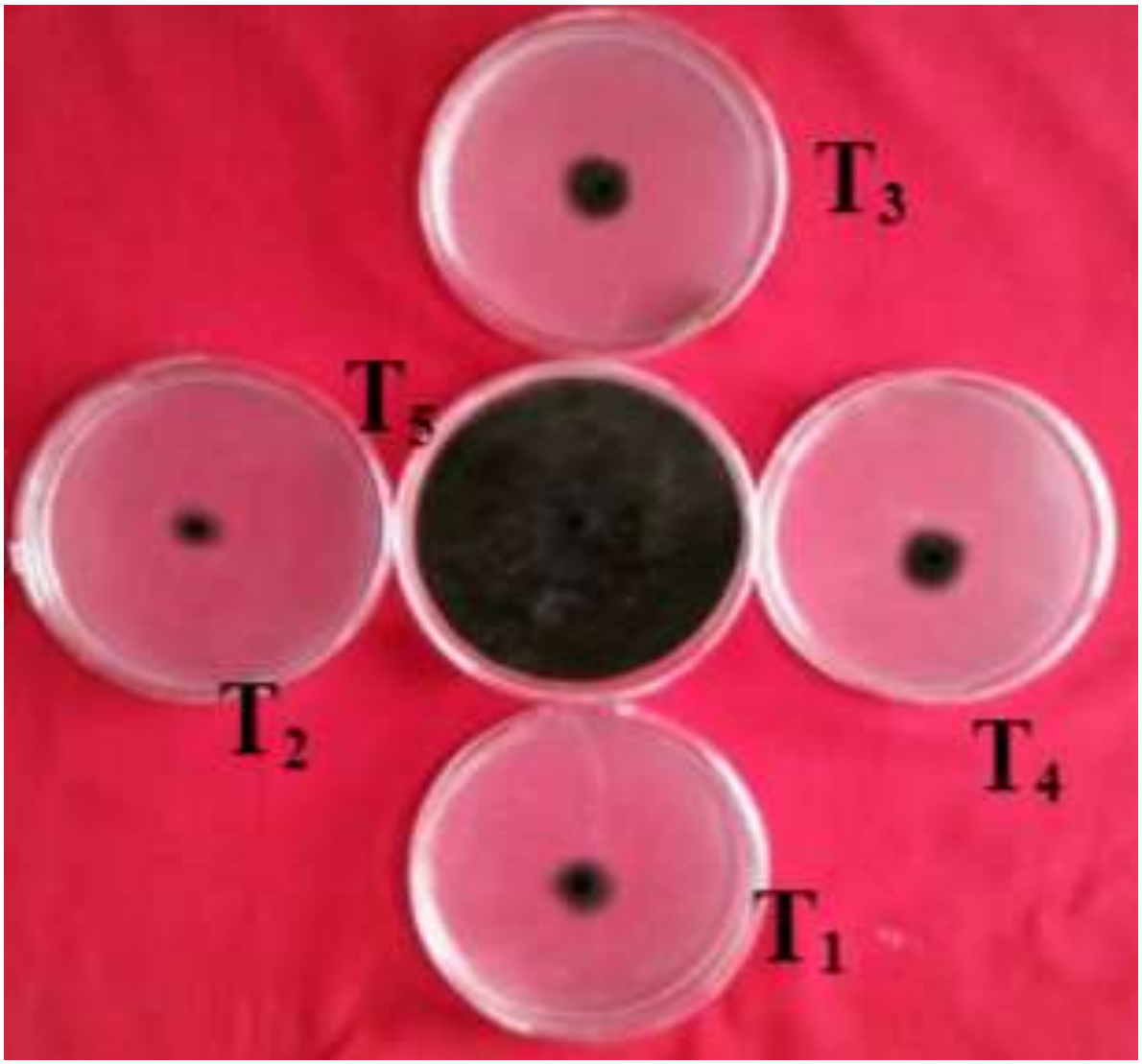
4.6 In-vitro efficacy of chemical against *Aspergillus niger*

Efficacy of four chemicals viz., Boric acid, Calcium chloride, Potassium chloride and sodium bicarbonate were tested using Poisoned Food Technique (PFT) for inhibition of mycelial growth of *Aspergillus niger*. Each chemical at concentrations of 1.0% were tested separately for inhibition of mycelial growth. Results obtained on mycelial growth inhibition of Aspergillus niger with tested chemicals are presented in the Table 7 and depicted in the Fig. 4. At 1% concentration of different chemicals, radial mycelial growth of *Aspergillus niger* was recorded against 89.00 mm in untreated control. However, significantly least mycelial growth was observed by recording maximum inhibition of with Calcium chloride which was (97.75%) at par with treatment of Boric acid (92.86%), while least mycelial inhibition was recorded with Sodium bicarbonate (87.05%) and Potassium chloride (86.54%).

Table 7. In vitro efficacy of chemicals against *Aspergillus niger*

Tr. No.	Treatment	Colony Dia * (mm)	Per cent Inhibition
T_1	Boric acid	6.23	92.86
T_2	Calcium chloride	2.05	97.75
T_3	Potasium chloride	11.78	86.54
T_4	Sodium bicarbonate	11.55	87.05
T_5	Control	89.00	00.00
	SE± CD @ 1%	00.83 3.26	

Fig.4



In vitro efficacy of chemicals against A*spergillus niger*

4.7 Effect of pre and post infection treatments of plant extracts on severity of tomato fruit rot caused by *Aspergillus niger*

Effectiveness of plant extracts were evaluated against fruit rot of tomato caused by *Aspergillus niger* by pre and post infection treatment method. It was revealed from data presented in Table 8 that all botanicals were found significantly superior in controlling fruit rot severity incited by *Aspergillus niger* on 8th day over control (80.04%). Among all pre infection fruit dip treatments, calcium chloride was found most effective and recorded least fruit rot severity on 8th day.

Table 8. Effect of pre and post inoculation treatment of plant extracts and chemicals on intensity of tomato fruit rot caused by *Aspergillus niger*

Tr. No.	Treatment	Pre inoculation	Post inoculation
T_1	Boric acid	65.34	15.01
T_2	Calcium chloride	61.28	14.23
T_3	Garlic	78.22	15.25
T_4	Coconut oil	79.13	23.54
T_5	Control	80.04	77.12
	SE ± CD @ 1%	00.74 3.15	00.53 2.23

Fig 5

Pre inoculation treatment with Mustard oil

Pre inoculation treatment with Cconut oil

Pre inoculation treatment with Sesamum oil

Pre inoculation treatment with Garlic clove extract

Pre inoculation treatment with Ginger rhizome extract

BEFORE TREATMENT

Fig 6

Pre inoculation treatment with Mustard oil

Pre inoculation treatment with Cconut oil

Pre inoculation treatment with Sesamum oil

Pre inoculation treatment with Garlic clove extract

Pre inoculation treatment with Ginger rhizome extract

AFTER TREATMENT

Fig 7

Post inoculation treatment with Mustard oil

Post inoculation treatment with Cconut oil

Post inoculation treatment with Sesamum oil

Post inoculation treatment with Garlic clove extract

Post inoculation treatment with Ginger rhizome extract

BEFORE TREATMENT

Fig 8

Post inoculation treatment with Mustard oil

Post inoculation treatment with Cconut oil

Post inoculation treatment with Sesamum oil

Post inoculation treatment with Garlic clove extract

Post inoculation treatment with Ginger rhizome extract

AFTER TREATMENT

DISCUSSION

Tomato (Lycopersicon esculentum Mill.) is a one of the important vegetable crop belongs to Solanaceae family. It is perennial in growth habit, however usually cultivated as an annual. It ranks second after potato in world acreage and is the first processing crop amongst all. It is originated from Tropical America (Bose *et al*., 1986) however domesticated and cultivated first in Central America. Now it is widely cultivated throughout the year under green house condition. Tomato is cultivated both in kharif and rabi season.

Kutama *et al.* (2007) observed 200 fungal isolates associated with tomato rots and classified into 5 genera namely *Aspergillus spp., Alternaria spp., Rhizophus spp., Penicillium spp. and Saccharomyces spp*. during the survey of Rimi market, Kanometropolis of Nigeria region.

Chavan (2012) isolated nine fungi viz., *Alternaria solani, Alternaria alternata, Alternaria citri, Aspergillus niger, Collectotrichum gloeosporioides, Fusarium oxysporum, Syncephalastrum racemosum* and *Cunnighhamella elegans* from rotten tomato fruits collected from different markets of Konkan region of Maharashtra state and recorded the highest frequency of A. solani in infected samples of tomato.

Sajad *et al.* (2017) found that most of the tomato fruits were suffered by fruit rot diseases caused by *Alternaria alternata, Aspergillus niger, Geotrichum candidum, Alternaria solani, Mucor racemosus, Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme, Penicillium digitatum, Rhi:opus stolonifer, Colletotrichum lycopersici, Sclerotium rolfsii, Myrothecium roridum, Phoma destructiva* and *Trichothecium roseum*. Whereas, *Alternaria alternata* (16.51%) had highest frequency of occurrence followed by *Alternaria solani* (12.43%), *Geotrichum candidum* (10.66%), *Aspergillus niger* (8.82%) and *Colletotrichum lycopersici* (7.53%) in local markets of Jabalpur district of Madhya Pradesh.

Bengerth *et al.* (1972) reported that post-harvest treatment with calcium was found effective in extending the storage life of many fruits by maintaining their firmness and minimizing the rate of respiration, protein break and rotting incidence.

Meena *et al*. (2006) evaluated fungicides and plant extracts for control of *Alternaria* fruit rot of brinjal and found that all fungicides and plant extracts were significantly superior over control on 4th and 7th day of pre and post inoculation treatment. Bavistin (0.1%) among fungicide gave lowest disease severity followed by raxi and inofil, whereas among plant extracts *Ocimum sanctum* leaves extract gave lowest disease incidence. A good control of *Fusarium* fruit rot of tomato was achieved by using *Azadirachta indica*, *Allium sativum*, *Ocimum sanctum* and *Curcuma longa* (Singh and Jain, 2007).

Aspergillus niger developed black rot with watery secretion and emittence of odour. Sinha and Saxena (1997) also observed the similar characteristics of symptoms on tomato fruit. They also found to produce similar types of black rots on number of vegetables and fruit crops (Muhammad *et al*, 2004, Gautam et al., 2010). Onuorah and orji (2015) described watery secretion and emittence of odor nature of spoilage by Aspergillus niger in tomato.

Effectiveness of plant extracts were evaluated against fruit rot of tomato caused by *Aspergillus niger* by pre and post infection treatment method. It was revealed from the data, that all botanicals were found significantly superior in controlling fruit rot severity incited by *Aspergillus niger* on 8th day over control.

Among all pre infection fruit dip treatments, garlic clove extract and coconut oil were found most effective along with Calcium chloride and boric acid.

SUMMARY AND CONCLUSIONS

Tomato (Lycopersicon esculentum Mill.) is one of the important and widely grown vegetable crop of Odisha state. The crop is affected by many fungal, bacterial, nematode and viral diseases. Among various problems, post-harvest fruit rot caused by fungal pathogen and their management is one of the important aspects to save produce from post-harvest losses. So an inspection on post-harvest fungal rot of tomato and its management was carried with reference to identification of various fungi associated with fruit rot of tomato, pathogenicity of isolated fungi, study of different symptoms produced by various associated fungi, efficacy of plant extracts and chemicals against major fungi associated with fruit rot of tomato, its post-harvest management and impact of post-harvest treatment of effective plant extracts and chemicals on marketable fruits and their spoilage.

The isolation and identification of the isolated fungi from the rotten tomatoes was done in the laboratory which were collected from different markets of Bhubaneswar region. Mycological analysis of rotten tomato yielded one species of Aspergillus genus i.e *Aspergillus niger* associated with the rotten tomato. The isolation process is done through standard tissue isolation technique. The well isolated individual colonies of fungus were reisolated on PDA media and slants. The isolates were identified and confirmed as *Aspergillus niger*.

The pathogenicity test was conducted with pin prick method where Aspergillus was inoculated into the healthy tomato fruits and had the same features as the one reisolated from them, indicating the fungi was responsible for rotting of the tomato fruit. The isolated fungi was found to be pathogenic which produced various lengths of rots after artificial inoculation. The isolates of *Aspergillus niger* developed black rot with watery secretion and emittence of odour

All tested botanicals were found to be effective against *Aspergillus niger*. Garlic clove extract and coconut oil was found most effective in inhibiting the mycelial growth of *Aspergillus niger*. All the tested chemicals were found fungistatic against Aspergillus niger. Calcium chloride and Boric acid at concentration 1% was found most effective in inhibiting the mycelial growth of *Aspergillus niger* while least

inhibition of these fungi was observed with Potassium chloride and Sodium bicarbonate.

Post-harvest treatments of chemicals by pre and post infection method showed the effectiveness Calcium chloride (0.2%) in controlling fruit rot of tomato followed by Boric acid (0.2%) against Aspergillus niger. Among plant extracts tested, ginger extract (10%) and seamum oil 2% were least effective but rather effective than nontreated fruits in controlling fruit rot of tomato. The post-harvest treatment of freshly harvested tomato fruits with Boric acid recorded minimum spoilage and PLW percentage than other treatments and untreated fruits.

CONCLUSIONS:

Thus, the results obtained from various aspects during present investigation on ‘‘**Microbial Association with harvested tomato fruits & its characterization**’’ it could be concluded that tomato fruit rot caused by *Aspergillus niger* is a major contributing post-harvest fungal fruit rot responsible for major losses.

Sample survey and collection of fruit rot affected tomatoes takes place during the month of January 2020 from different markets of Bhubaneswar region.

Pathogenicity test was conducted by using pin prick method and the isolates of fungus *Aspergillus niger* was found pathogenic causing post-harvest tomato fruit rots.

The isolates of *Aspergillus niger* produced typical symptoms after artificial inoculation as black to dark rots on fruit more often with watery secretion and emittence of odour character. Finally causing the fruits to completely decayed.

Aqueous extract of botanicals viz., garlic, Ginger along with mutard, coconut and sesamum oil were found fungistatic against *Aspergillus niger*. These botanicals further used to be tested for economical and eco-friendly management of *Aspergillus niger*.

All the 4 chemicals tested in-vitro were found to inhibit mycelial growth of, *Aspergillus niger*, However, calcium chloride and boric acid were highly effective against *Aspergillus niger* as compared to all other treatments.

Fruit dip with Post inoculation method gave least fruit rot intensity with Calcium chloride and boric acid as compared to pre inoculation fruit dip method, whereas ginger extract and sesamum oil were least effective in controlling fruit rot caused by post-harvest fungi.

Post-harvest treatment of harvest fruit with Calcium chloride and Boric acid gave least spoilage and physiological weight loss.

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