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Evaluation of Antifungal Properties of Botanicals in Green Mould of Citrus

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ABSTRACT

The most economically important post harvest disease in citrus is considered to be Green mould disease of citrus caused by *Penicillium digitatum*. In an attempt to explore the possibilitiesto develop effective post harvest management practices and keeping in mind the harmful effects of the use of chemicals ten botanicals *viz. Azadiracta indica, Capsicum annum, Eucalyptus citriodora* Hoch., *Zingiber officinalis, Allium sativum, Murrya koenigii, Bacopa onneiri, Zanthoxylum oxiphyllum* DC., *Allium cepa* and *Mentha piperita* L. were screened under *in vitro* and *in vivo* conditions for their inhibitory action on the mycelial growth of *Penicillium digitatum*. Among the botanicals screened, *Allium sativum* recorded maximum per cent inhibition (76.89%) at 15% concentration which was followed by *Capsicum annum* (68.94%) at 15% concentration. Three best anti-fungal activity plant crude extracts based on *in vitro* screening results were evaluated to check their efficacy under *in vivo, viz., Allium sativum, Mentha piperita* and *Capsicum annum* against *Penicillium digitatum* under pre inoculated and post inoculatedconditions. It was found that *A. sativum* recorded the least disease incidence with PDI of 24.67% and 34.67% under pre inoculated and post inoculated condition followed by *Mentha piperita* recording PDI of 28.00% and 34.00% under pre inoculated and post inoculated conditions.

1. Introduction

Citrus (*Citrus sinensis* L.) occupies a prominent place of commerce among the tropical and sub tropical fruits crops that is produced globally. *Citrus sinensis* L. is a native of Asia, mainly South-east Asia and belongs to the family Rutacea (Ojo, 2014). It is cultivated mainly in the subtropical and tropical regions, over 137 countries on six continents (Ismail and Zhang, 2004). The importance of citrus fruit is attributed to its widely use, which is consumed as fresh fruit and also as juice (Talibi, 2014).

Inspite of containing good source of vitamin C, citrus fruits also contain important essential nutrients such as glycamic and non-glycamic carbohydrate (sugars and fibre), potassium, folate, calcium, thiamin, niacin, vitamin B_6, phosphorus, magnesium, copper, riboflavin, pantothenic acid and phytochemicals. Majorly citrus is dominated by sweet orange (71%) followed by mandarins (13%), limes and lemons (10%) and the rest 6% is bestowed by grapefruit and others. Brazil produces 1/4th of the world's citrus, of which75% is processed for juice.

In India, 26 states are undergoing citrus cultivation in which 9 of them are contributing more than 70% of area and 89% of total citrus production. In India limes/ lemons occupies an area of 1,28,006 ha, 10,51,835 tons production and 8.0 tons/ha productivity while mandarin cultivation contribute to 14, 72,401 tons production with 1,65,376 ha area and 9.0 tons ha^{-1} as productivity (Anon. 2014).

The North Eastern States of India considered as a repository for a number of citrus species. Constituted a total of 17 species, 52 varieties and 7 hybrids which have been reported from this region, citrus is one of the important fruits of North Eastern States and occupied third in accordance to area and production. It has been grown in an area of about 19,117 hectares, amounting an annual production of 119,749 tones. Many citrus species has been identified in this region and later distributed to today's frontline citrus growing countries of the world. 'Soh-nairing' and 'Soh- bitara' of Meghalaya and 'Tasa' of Arunachal Pradesh are important indigenous cultivars of the *Citrus sinensis* group. Among the

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North Eastern states, Meghalaya occupied the leading state in both area and production which is succeed by Manipur, Assam, Tripura, Mizoram, Nagaland, Arunachal Pradesh and Sikkim (Singh, 2001). In Nagaland citrus occupied an area of 61000 ha amounting an annual production of 548000 MT. The climate of citrus growing areas in Nagaland is predominantly humid having a mean rainfall of 2000mm. The maximum varies from 18.2^{o}C and the minimum temperature ranges from 7.2^{o}- 18.1^{o}C, respectively with 52-86% relative humidity (ICAR complex for NEH Region, Medziphema, Nagaland).

Citrus fruit is found in abundance during the season. During the period between harvest and consumption, citrus fruit is found susceptible to microbial pathogens infection due to its higher water content and nutrient composition (Tripathi and Dubey, 2003). Citrus fruits having a pH range of 2.2-4 are quite acidic and for this reason most of the post harvest losses is caused by fungi. It has been reported that one of the most limiting factors that influence the fruits economic value is the relatively short shelf life caused by fungi and also 50% of the loss was due to poor storage conditions (Birhanu *et al.,* 2014). Major postharvest losses have been found to be associated with a range of fungi such as *Penicillium digitatum, Penicillium italicum, Aspergillus niger, Monilinia lax,* and *Rhizopus stolonifer* which were recorded in association with post-harvest diseases (Ogawa *et al*., 1995). Among the different diseases that threaten citrus crops, citrus green mould disease (*Penicillium digitatum*) causes the most economically important postharvest disease (Mekbib *et al.,* 2009). The primary infections of the pathogen takes place only through wounds on fruit when nutrients are available to stimulate the germination of spores. Wounds are caused by poor postharvest handling practices during picking, and handling. Initial infections appear as water soaked, soft areas easily punctured by finger pressure. The area enlarges and on the fruit surfaces white mycelium appears along with olive green spore mass containing 100, 000,000 spores in ten days (Bajwa, 2007).

In order to reduce postharvest losses, preservation of fresh fruit, control of postharvest decay, and extend its shelf life, postharvest treatments with conventional synthetic waxes and/or chemical fungicides are still currently in used. Although, the continuous used of such treatments led to important problems for the citrus industry and the commercial use of postharvest chemicals has become restricted because of public health concerns (Unnikrishnan and Nath, 2002), development of pathogen resistance (Fogliata *et al*., 2001; Dianz *et al*., 2002) and environmental issues. Therefore, there is an need to develop an alternative postharvest management strategies involving biological agents or natural plant extracts which is being an environmentally safer and more acceptable to the general public (Janisiewicz and Korsten, 2002).

Traditionally it has been recorded the use of plant extracts for the control of plant diseases (Ark and Thompson, 1959). The actual reason for the role of plant extracts in plant disease control is still lacking (Obagwu, 2003). Plant extracts are preferable as one of control measures because of their anti-fungal activity, non-totoxicity, systemic in action and also biodegradability (Askarne *et al.,* 2012). Therefore, there is a requirement to identify the most effective plant extracts for the management of postharvest decay and determine the disease incidence caused by the *Penicillium digitatum* Sacc. causing citrus green mould disease.

2. Materials and Methods

Isolation, Identification and Pathogenicity test of P. digitatum

Diseased fruits of orange showing characteristic symptoms of green mould disease were collected from the citrus growers of Kohima Village, Meriema Village, Tuophema Village, Chiedema Village, Chiechama Village under Kohima district. The diseased specimens were further studied in the laboratory for isolation and the pathogen associated was identified.

The disease orange fruits were taken into the inoculation chamber and a diseased portion was sliced out from infected part and surface sterilized with 1% Sodium hypochloride for 60 seconds. These were then washed thoroughly three times with distilled water. Diseased parts were placed blot dried with the help of sterile blotting paper. On petriplates containing PDA medium, one slice of disease sample was placed at the centre. Incubation was done at 27 ± 1 °C in BOD. By following single hyphal tip methods, the pure culture of the pathogen was maintained and identification was made based on their morphological and cultural characteristics such as colony characters, mycelium, conidiophores and conidia.

Following Koch's postulates, the pathogenicity test was conducted by inoculating healthy fruits with the test pathogen *P. digitatum* under the laboratory. The fruits were surface sterilized with 1% Sodium hypochlorite, washed three times with sterile distilled water and then allowed for air drying followed by artificially pricked 1 mm depth by using sterile needle, and then inoculating the fruits with 10^6 conidial suspensions of *P. digitatum*. Healthy fruits inoculated with distilled water were maintained as control. Observations based on the occurrence of typical mould rot symptoms on the fruits after certain period of incubated in room condition was recorded. Artificial inoculation of pathogen, observations on development of disease symptoms and again reisolation was being recorded in order to confirm the pathogen *P. digitatum* as the causal organism of Green mould of citrus.

In vitro screening

Preparation of plant extracts

The selected plant part samples were brought in the laboratory and cleaned thoroughly in water. The plant samples (100 g) were individually ground in mortar and pestle. In a conical flask, soaked the plant extracts in ethanol @ 95% at a ratio of 1:1 w/v and allowed each sample to evaporate. After evaporation, add 100 ml of sterile distilled water into the plant extract in a conical flask and stirred it well with sterile stirring rod. The plant extracts was then filtered by using sterile filter paper and the sample thus obtained was considered as 100 % standard extract.

Effect of botanicals on radial growth of *P. digitatum*

Under *in vitro* condition, the selected plant extracts were screened against the pathogen by poisoned food technique as suggested by Nene and Thapliyal,1982. The required volume of plant extracts *viz.* 5, 10 and 15 percent was taken from the standard plant extract solution (100%) and mixed in sterilized molten PDA medium and then thoroughly mixed. 20 ml of PDA media amended with plant extract was poured in sterilized petriplates (90 mm) under aseptic condition. 7 days old culture of the test pathogen was used by transferring 5 mm disc cut of the pathogen with sterilized cork borer at the centre of the petriplates. Incubation was done at 27±1°C in BOD and observation was recorded at 96 hrs, 120 hrs, 144 hrs and 166 hrs.

In vivo screening

Effect of botanicals on radial growth of *P. digitatum*

The effects of botanicals were studied on susceptible citrus fruits against *P. digitatum* in pre inoculated and post inoculated conditions under room temperature. The experiment was conducted in Completely Randomized Design (CRD) in which three replication was maintained. Healthy fruits were washed thoroughly and then air dried and sterilization takes place by sodium hypochlorite. The test fruits after proper sterilization and cleaning were inoculated with the inoculum of *P. digitatum* by pin prick method. A set of 10 fruits were maintained for each treatment which represented a single replication and was replicated 3 times. A uniform dose of 15 ml each was applied in each treatment. In the pre inoculated treatments application of botanicals were done 12 hours prior to the inoculation of *P. digitatum.* In the post inoculated treatments, botanicals were applied 12 hour after the inoculation of *P. digitatum.* Based on the result of the test of efficacy of the botanicals against the pathogen in *in vitro,* 15 per cent concentration of the three most efficient crude extract were maintained. In control treatment, spraying of only sterilized water was done in both pre and post inoculated conditions. A hand sprayer was used for application of all the treatments. Observations on disease intensity were recorded at 3, 6, and 9 days after inoculation.

Table 1. List of the Botanicals

SL no.	Common name	Botanical Name	Family	Plant part used
1	Neem	*Azadiracta indica*	Meliaceae	Leaf
2	Chilli	*Capsicum annum*	Solanaceae	Fruit
3	Eucalyptus	*Eucalyptus citriodora*	Myrtaceae	Leaf
4	Ginger	*Zingiber officinale*	Zingibearaceae	Rhizome
5	Garlic	*Allium sativum*	Amaryllidaceae	Clove
6	Curry leaf	*Murrya koenigii*	Rutaceae	Leaf
7	Brahmi	*Bacopa onneiri*	Plantaginaceae	Leaf
8	Zanthoxylum	*Zanthoxylum oxyphyllum*	Rutaceae	Leaf
9	Onion	*Allium cepa*	Amaryllidaceae	Bulb
10	Mint	*Mentha piperita* L.	Labiatae	Leaf

Observations recorded

Percent inhibition (PI) of the pathogen (*P. digitatum*) was calculated based on Vincent, 1947.

PI = C-T/C× 100

Where, T = Growth of the pathogen in treatment C = Growth of the pathogen in control

Disease intensity

Observations on the disease intensity were recorded at 3, 6 and 9 days after inoculation. The intensity of fruit rot disease was recorded by adopting the following disease rating scale

Scale	Disease Reaction
0	Apparently healthy fruit
1	Slight infection
2	25% fruit area infected
3	50% fruit area infected
4	100% fruit area infected
5	Entire fruit infected

Therefore,

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{Total no.of fruits observed x maximum rating}} \text{x100}$$

3. Results and Discussion

Isolation and identification of *P. digitatum*

The pathogen causing green mould was isolated from orange fruits showing symptoms and identification was done based on the morphological character of the pathogen and the appearance of the symptoms on the fruit. The pathogen was confirmed to be *Penicillium digitatum*. The fungal colony appeared as white in colour which later turned dull to citrine green in colour due to sporulation. The mycelia were arranged very irregularly, conidia were smooth and cylindrical and present in long chains on the conidiophores which resemble a broom. On the fruits, initial infection appears as water soaked soft areas easily punctured by finger pressure. This area enlarges and white mycelium appears on the surface followed by olive green spore mass adherents the appearance of white mycelium around the rind surrounding the entire fruit. The findings was in corroborate with Birhanu *et al.* (2014) who observed that conidia of *P. digitatum* produced on branched conidiophores in a long chains which resemble brush-like head (*Penicillus*) were conidiophores are smooth and relatively short. Penicillia are arranged in an irregular and asymmetrical pattern with branches of variable lengths.

***In vitro* test**

Effect of botanicals on *P. digitatum*

Crude extract of ten botanicals *viz.* Neem, Chilli, Eucalyptus, Ginger, Garlic, Curry leaf, Brahmi, *Zanthoxylum* and Onion were screened for their inhibitory action against the mycelial growth and per cent inhibition of *P. digitatum*. The results presented in Table 2 (a) and 2(b) and Fig.a, which clearly indicates that a pronounced reciprocity in the per cent inhibition of *P. digitatum* with the application of higher concentration of the leaf extracts when compared with control. Amongst the different plant extracts that have been tested at concentrations 5%, 10% and 15%, the least mycelial growth with a maximum per cent inhibition of *P. digitatum* was recorded with 15% concentration at 96 hrs, 120 hrs, 144 hrs and 166 hrs of incubations. Among the botanical extracts Garlic (*A. sativum*) was observed to be the mostpromising against *P. digitatum* @ 15% concentration resulting in least mycelial growth of 20.33 mm with maximum per cent inhibition of 76.89% at 166 hrs. This was followed by application of 15% concentration of Chilli (*Capsicum annum*) 27.33 mm mycelial growth and 68.94% inhibition and Mint (*M. piperita*) with 35.33 mm and 59.85% respectively. Leaf extracts of Brahmi (*B. onneiri*) and Curry leaf (*M. koenigii*) on the other hand had the least effect on *P. digitatum* in all the treatment concentrations applied, at all days of observation recorded and it was observed that they did not effectively control *P. digitatum* as compared to other treatments. The observations showed that control treatment could not inhibit the growth of *P. digitatum* at any stage and recorded 0% inhibition throughout the incubation period. Among the three concentrations, the extracts at 15% concentration were significantly superior to 5% and 10%.

These above findings are in conformity with Afzal *et al.* (2010) who observed that *A. sativum* showed a wide antifungal spectrum and inhibited 60-82% of the radial growth of *P. digitatum.* Birhanu *et al.* (2014) also found out that aqueous and ethanolic extracts of *A.sativum* at 15% and 10% inhibited the growth of *Penicillium* causing green mould of citrus by 84.11% and 76.13% respectively. Daniel *et al.* (2015) also reported that concentration @ 80% of aqueous and ethanol exracts inhibited the radial growth of *P. expansum* on apples by 96.21% and 99.21% respectively. Antifungal activity of plant extracts against various fungi has been reportedby various workers. Singh and Sumbali (2003) reported that leaf extracts of *Mentha piperita, M. spicata* and *M. longifolia* at 25% concentration were quite effective in arresting the development of *P. expansum* rot of apples. Erui (2009) also reported that mint leaf extracts at 15% concentration showed significant effect on the mycelia growth and per cent inhibition on *P. islandicum* on aonla.

Singh *et al.* (2011) reported that *Capsicum frutescence* showed 100% inhibition at 3000 ppm against *P. digitatum* causing green mould of citrus. Garlic contains allicin (allyl 2-propenethiosulfinate or diallylthiosulfinate) which is the principal bioactive component present in the aqueous solution of garlic or raw garlic homogenate. Other important compounds present in garlic homogenate are 1 -propenyl allyl thiosulfonate, allyl methyl thiosulfonate, (E,Z)- 4,5,9-trithiadodeca- l,6,11-triene 9- oxide (ajoene), and y-L-glutamyl-S-alkyl- L- cysteine (Bayan *et al.* 2014). The garlic plant contains these steroidal alkaloids which may have retarded the growth of the pathogen. The inhibitory effect of different leaf extracts on mycelial growth of *P. digitatum* in the present investigation may be attributed to the presence of steroidal alkaloids.

***In vivo* evaluation**

Under *in vivo* experiment the best three anti-fungal activities of botanicals based on *in vitro* screening results were evaluated *viz. Allium sativum, Capsicum annum* and *Mentha piperita*. The effects of the selected botanicals were studied under pre inoculated and post inoculated conditions. The observation was recorded in Table 3 and Table 4 and Fig b. Initiation of the disease under both pre and post inoculated conditions were recorded after three days of inoculation (DAI). From the values provided in Table 3 and Table 4, it was found that the intensity of the disease gradually increased with the progress in time. The disease intensity was observed to be maximum in the pre inoculated treatments as compared to the post inoculated treatments which evidently indicate that treatments were more effective when applied as pre inoculation than post inoculation.

It was recorded that when the treatments were applied as pre inoculated it was observed to be more effective than the post inoculated treatments. Of the three botanicals tested, crude extract of *A.sativum* observed the least disease incidence with PDI of 24.67 and 34.00 under pre inoculated condition and post inoculated condition respectively. The results were however statistically at par with *Mentha piperita* recording PDI of 28.00 and 34.67 under pre inoculated and post inoculated condition respectively followed by PDI of 31.33 in pre inoculated condition and 40.67 in post inoculated condition in *C. annum*.

Perusal of the results revealed that amongst the treatment *Allium sativum* was found to be the most promising in controlling *Penicillium digitatum*, however found to be statistically at par with *Mentha piperita* and *Capsicum annum.* A similiar finding was reported by Birhanu (2014) who reported that aqueous extracts (15%) of *A. sativum* were effective in reducing the disease severity against *P. digitatum* of citrus. Obagwu and Korsten (2003) reported aqueous (10g in 50 ml of water) and ethanol extracts (10 g in 50 ml of 20% ethanol) of *A. Sativum* controlled *P.digitatum* causing green mould of citrus. Erui (2009) reported that leaf extracts of mint showed significant effect on *P. islandicum* causing blue mould of aonla. Al Samarrai (2013) reported that crude extracts of chilli remarkably reduced the undesirable fruits percentage, fruit weight loss percentage during storage.

In the present study, water extracts of the botanicals were used and hence only the water soluble portions of the components present in the leaf of the botanicals encountered with the disease. The variation in the efficacy of the botanicals in controlling different crop diseases has been documented by differentworkers (Singh and Sumbali, 2003).

Results showed that the treatments had an effective

Under both pre inoculated condition and post inoculated condition, *C. annum* showed maximum weight loss on the sixth day measuring 188.13 g and 174.22 g .On the ninth day maximum weight loss was found on *A. sativum* under post inoculated condition measuring 126.79 g and under pre inoculated condition maximum weight loss was found to be in *C.annum* measuring 155.74 g (Table 5 and Table 6). Results showed that garlic (*A. sativum*) showed the best result in reducing the weight loss of the fruit under pre inoculated condition and Mint (*M. piperita*) showed the best result in reducing the weight loss of the fruit under post inoculated condition. This agrees with the finding of Al Samarrai *et al.* (2013) who opined that fruit loss per cent is due to loss in moisture content and the disease infestation on the fruits. Increased weight loss with increasing storage is common in fruits.

4. Conclusions

From the research findings, it can be concluded that although application of the three botanicals *viz. A. sativum, C.annum and M. piperta @* 15% concentration were found to be significantly superior to all the botanicals that were tested in controlling green mould of citrus, crude extracts of *A.sativum* showed significant decrease in the growth rate of the pathogen and it was either superior or statistically *at par* with the other treatments. The efficacy of the botanicals was found more potent when applied as preinoculated treatment as post inoculated treatment. However, considering the large number of bioactive chemicals present in plant extracts, it is most likely that their antimicrobial activity is not attributable to one specific mechanism but to diverse mode of action. Further investigation on the useof leaf/stem/peel extract in organic solvent as well as use of essential oils of botanicals will certainly elucidate some additional results. Moreover, it is expected to generate useful information by testing larger

number of plants/plant parts in plant disease control in general and green mold in particular. This expectation may be many folds as Nagaland and North East India as a whole is known as biodiversity hot spot.

Table 2 (a). Effect on botanicals on radial growth of *Penicillium digitatum*

Treatment		Per cent inhibition of *P. digitatum* in presence of botanicals											
		96hrs			120hrs			144 hrs			166hrs		
		5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%
T_1	*P. digitatum* + Neem (L)	32	31	23.33	40.17	35.33	29.17	52.17	43.50	37.33	67.50	51.17	42.67
T_2	*P. digitatum* + Chilli (F)	23.33	17.67	15.5	30.33	24.33	19.83	36.83	28	23.33	46.33	34.67	27.33
T_3	*P. digitatum* +Eucalyptus (L)	31.50	28.33	21.83	36.33	34.33	26.33	49.33	39	34.67	64	48.33	38.33
T_4	*P. digitatum* + Curry leaf (L)	45	43	37.67	60.33	61.33	42.67	70	65.67	47.67	81.33	81.83	55
T_5	*P. digitatum* + Mint (L)	28.67	24.67	17	33.33	28.33	21.67	48.33	34.33	27	58.50	40.67	35.33
T_6	*P. digitatum* + Ginger (R)	43.33	41.67	35	56.67	54.83	39.5	66.83	64.67	44.67	78.50	69.67	49.67
T_7	*P. digitatum* + Garlic (C)	18.33	15	12.33	25.67	18.33	14.33	34.83	23.33	16.67	39	27.67	20.33
T_8	*P. digitatum* + Brahmi (L)	47	44.67	39.67	63.67	63.33	47.67	77	74.67	52	85.33	84	60
T_9	*P. digitatum* + Zanthoxylum (L)	38.33	34.33	25.17	43.17	38.33	31.33	55.33	44.33	38.33	61.33	52.33	44
T_{10}	*P. digitatum* + Onion (B)	23.33	17.67	15.5	42.67	38.67	33.67	54.67	46.67	39.17	69.67	54.67	45.83
T_0	Control	64.33	64.33	64	78	78	78	85	85	85	88.33	88	88
		Trt.	Conc.	Trt× Con	Trt.	Conc.	Trt× Con	Trt.	Conc.	Trt× Con	Trt.	Conc.	Trt× Con
SEm±		0.34	0.18	0.58	0.41	0.21	0.71	0.39	0.20	0.68	0.26	0.13	0.44
CD(p=0.05)		1.09	0.57	1.89	1.33	0.69	2.30	1.26	0.66	2.19	0.83	0.43	1.44

Table 2 (b). Percent Inhibition of *P digitatum* in presence of botanicals at different concentrations:

Treatment		Per cent inhibition of *P. digitatum* in presence of botanicals											
		96hrs			120hrs			144 hrs			166hrs		
		5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%
T_1	*P. digitatum* + Neem (L)	50.24	51.84	63.54	48.50	54.70	62.61	38.63	48.82	56.08	23.58	41.86	51.52
T_2	*P. digitatum* + Chilli (F)	63.73	72.53	75.78	61.11	68.80	74.57	56.67	67.06	72.55	47.54	60.61	68.94
T_3	*P. digitatum* +Eucalyptus (L)	51.03	55.95	65.89	53.42	55.98	66.24	41.96	54.12	59.22	27.55	45.08	56.44
T_4	*P. digitatum* + Curry leaf (L)	30.04	33.17	41.15	22.65	21.37	45.30	17.65	22.75	43.92	7.92	7.01	37.5
T_5	*P. digitatum* + Mint (L)	55.43	61.65	73.44	57.26	63.68	72.22	43.14	59.61	68.24	33.77	53.79	59.85
T_6	*P. digitatum* + Ginger (R)	32.64	35.23	45.31	27.35	29.70	49.36	21.37	23.92	47.45	11.13	20.83	43.56
T_7	*P. digitatum* + Garlic (C)	71.51	76.68	80.73	67.09	76.50	81.62	59.02	72.55	80.39	55.86	68.56	76.89
T_8	*P. digitatum* + Brahmi (L)	26.93	30.57	38.02	18.38	18.80	38.89	9.41	12.16	38.82	3.39	4.55	31.82
T_9	*P. digitatum* + Zanthoxylum (L)	40.42	46.63	60.68	44.66	50.85	59.83	34.90	47.84	54.90	30.56	40.53	50
T_{10}	*P. digitatum* + Onion (B)	45.59	49.22	56.77	45.30	50.43	56.84	35.69	45.10	53.92	21.13	37.88	47.92
T_0	Control	0	0	0	0	0	0	0	0	0	0	0	0
		Trt.	Conc.	Trt× Con	Trt.	Conc.	Trt× Con	Trt.	Conc.	Trt× Con	Trt.	Conc.	Trt× Con
SEm±		0.53	0.28	0.91	0.52	0.27	0.91	0.46	0.24	0.79	0.3	0.16	0.53
CD(p=0.05)		1.71	0.89	2.96	1.70	0.89	2.95	1.49	0.78	2.58	0.99	0.52	1.71

Table 3. Efficacy of botanicals under pre inoculated conditions

Treatment	Percent disease index at diferent days of interval		
	3 DAI	6 DAI	9 DAI
T_1 (Mint- *M. piperita)*	4.00	12.67	28.00
T_2 (Chilli- *C. annum)*	8.00	14.00	34.00
T_3 (Garlic- *A. satiivum*)	3.33	18.00	24.67
T_4 (Control)	22.00	46.00	69.33
SEm±	0.75	1.45	2.58
CD(p=0.05)	2.43	4.74	8.42

Table 4. Efficacy of botanicals under post inoculated conditions

Treatment	Percent disease index at diferent days of interval		
	3 DAI	6 DAI	9 DAI
T_1 (Mint- *M. piperita)*	6.67	16.67	34.67
T_2 (Chilli- *C. annum)*	11.33	25.33	40.67
T_3 (Garlic- *A. satiivum*)	5.33	21.33	34.00
T_4 (Control)	22.00	58.00	84.67
SEm±	1.41	1.63	3.37
CD(p=0.05)	4.61	5.33	10.98

Table 5. Weight loss of the fruits under pre inoculated conditions

Treatment	Weight of the fruits (g)			
	Initial	3	6	9
T_1 (Mint- *M. piperita)*	283.10	223.15	121.11	97.28
T_2 (Chilli- *C. annum)*	412.67	339.05	188.13	155.74
T_3 (Garlic- *A. satiivum*)	209.96	191.04	152.12	108.06
T_4 (Control)	249.84	222.99	154.27	167.19
SEm±	15.39	13.71	5.23	15.84
CD(p=0.05)	50.19	44.70	17.05	51.67

Table 6. Weight loss of the fruits under post inoculated conditions

Treatment	Weight of the fruits (g)			
	Initial	3	6	9
T_1 (Mint- *M. piperita)*	231.30	197.32	117.06	93.67
T_2 (Chilli- *C. annum)*	366.32	297.18	174.22	135.17
T_3 (Garlic- *A. satiivum*)	208.82	196.27	166.84	126.79
T_4 (Control)	299.39	275.79	241.68	191.30
SEm±	21.82	19.11	12.74	10.80
CD(p=0.05)	71.16	62.33	41.55	35.22

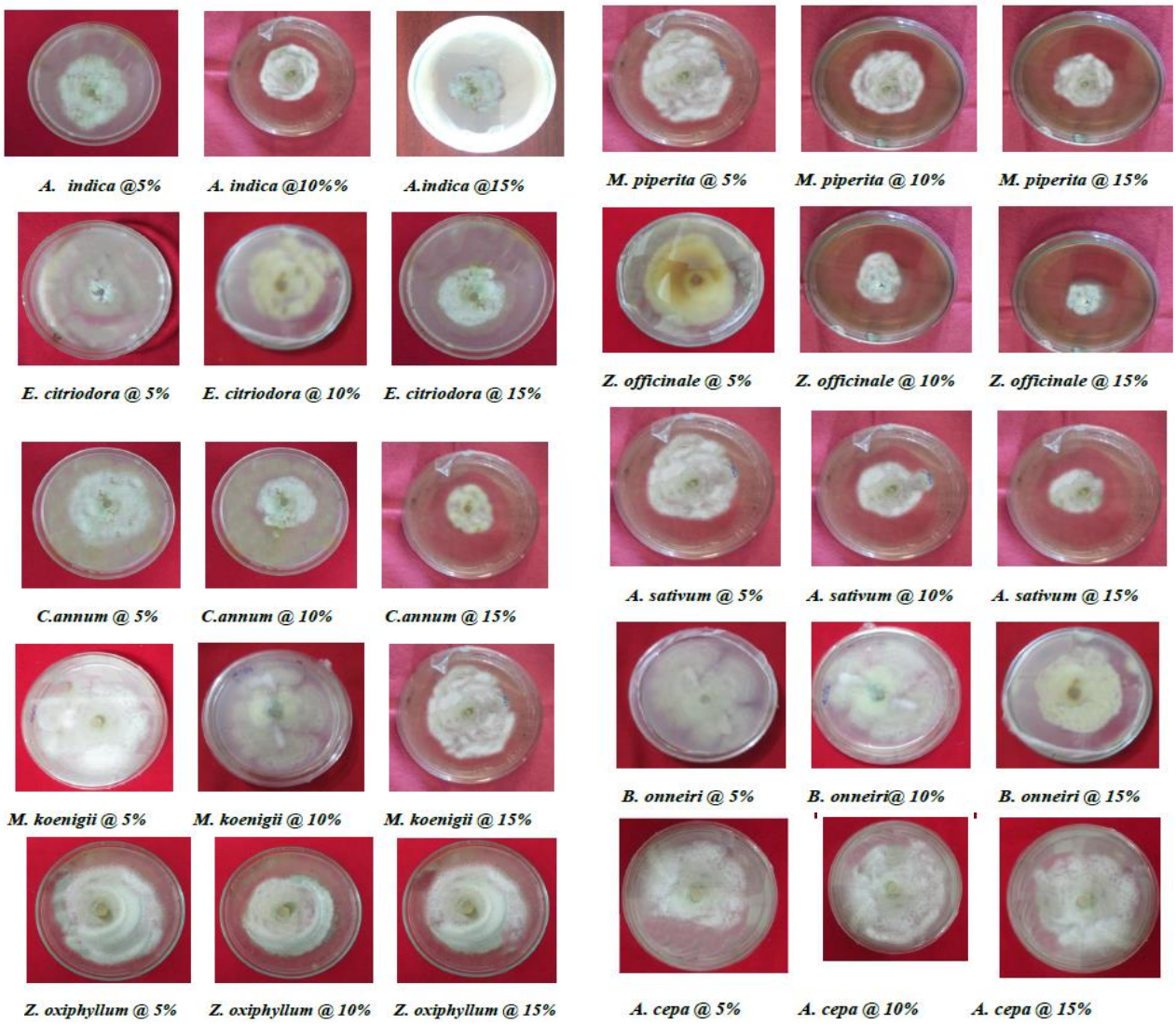

Figure a. Inhibitory effect of Botanicals on *P. digitatum*

Control plate

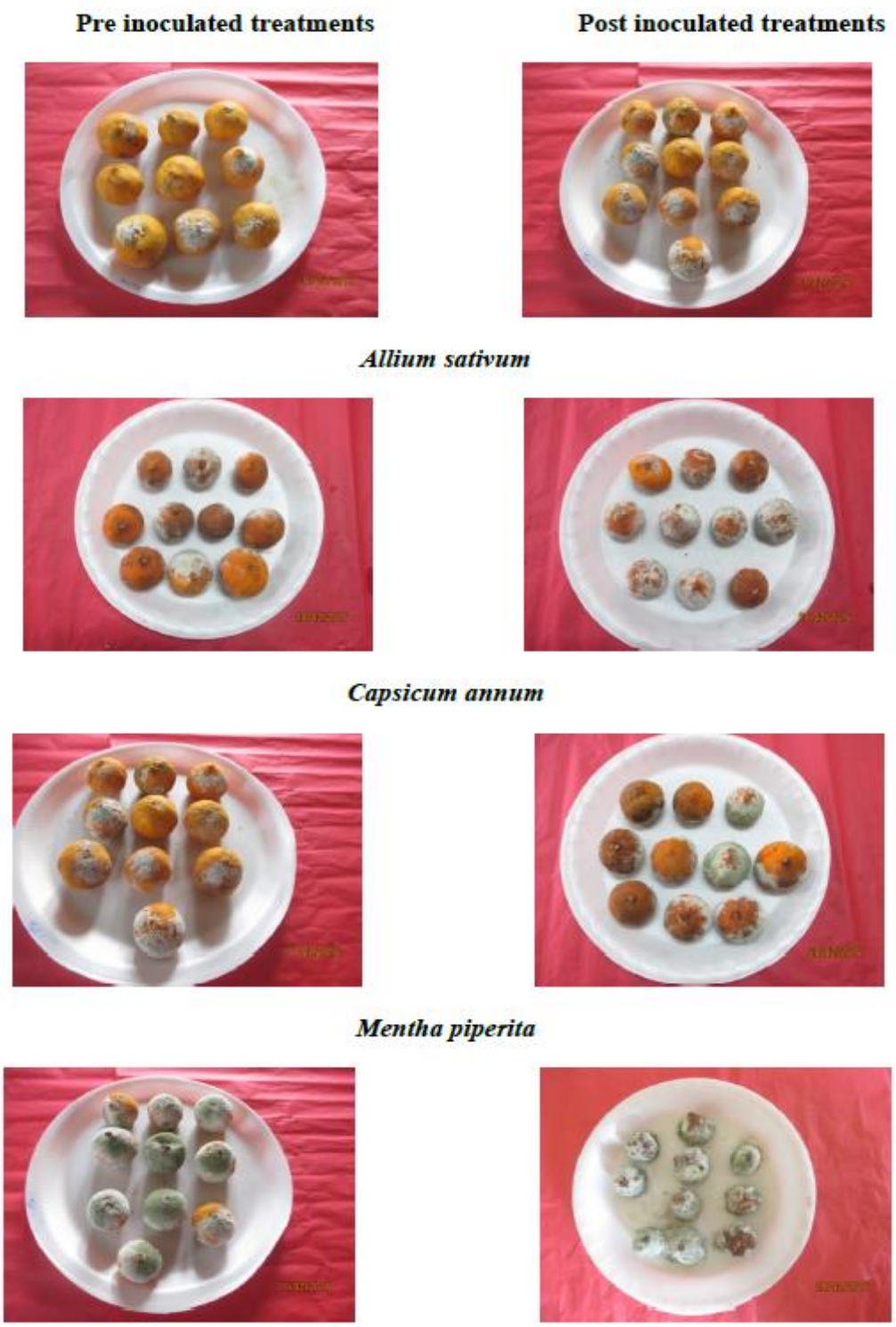

Figure b. Evaluation of selected Botanicals against P.digitatum

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