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Effect of different plant extracts and their antimicrobial properties against *Penicillium expansum* and shelf life of apple

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Abstract
Six crude plant extracts of lantana, tridax, coleus, lemongrass, neem and tulsi were evaluated against *Penicillium expansum* the cause of blue mould disease of apple was evaluated for their inhibitory effect both *in vitro* and *in vivo* during the storage condition. Shelf life of apple was also evaluated while using this plant extracts. The concentration of 5000 ppm from ethanolic extract of tulsi showed highest inhibition of pathogen and it reached 71.11% followed by neem 66%. Then minimum weight loss percent and TSS percent was shown by apple treated with ethanolic tulsi extract at 5000 ppm concentration. *In vivo* experiment treating fruits with different plant extracts at 5000 ppm concentration showed reduced lesion diameter in apples treated with *Penicillium expansum*. Maximum shelf life in apple was shown by fruits treated with ethanolic tulsi extract at 5000 ppm concentration upto 14 days. Then qualitative phytochemical analysis of ethanolic tulsi extract was carried out and it showed presence of different phytochemicals. Plant extracts was having remarkable usage in future against pathogen and it will surely replace synthetic fungicide usage.

Keywords: Plant extracts, *Penicillium expansum*, antimicrobial, shelf life, apple

1. Introduction
India's distinct climatic condition ensures availability of many types of fruits. India ranks second in fruit production in the world, followed by China. India produced 90.2 million MT of fruits during 2015-2016. Fruits were required for a healthy diet, because it contains high amount of vitamins, minerals and antioxidant compounds. Unfortunately, fruits are more perishable in nature during their postharvest days and fungal infection leads to loss of fifty percent of total production in many countries (LiDestri *et al*., 2016) [21]. India has enormous capacity for the production of various kinds fruits and also their export (Yahaya *et al*., 2019) [29]. The fruits that are consumed and exported on large scale includes apple, grapes and orange. Postharvest losses of vegetables and fruits can reach very high values, representing more than 25% of the total production in industrialized countries and more than 50 percent global, if postharvest handling and storage conditions are not optimal. In fruit majority of the losses are due to the attack of several fungal pathogens due to the high amount of nutrients and water content, low pH and loss of inherent resistance that protects them while they are attached to the plant (Droby *et al*. 1992) [10].

Apple is one of the globally important fruit. Due to its high nutritious properties, it ranks third in consumption after citrus and banana (Bokhari, 2002) [6]. Apples and their processed edible products have been consumed by everyone all over the world. It is having more nutritious value. The postharvest losses in apple may depend on both external and internal conditions. Among the external conditions major factors are temperature and moisture content during postharvest handling operations influencing the performance of apple during storage (LeBlanc *et al*., 1996) [20] Major postharvest disease of apple was cause by the pathogens such as Botrytis cinerea, Penicillium expansum and Monilinia fructigena, the causal agents of grey mould, blue mould, and brown rot, respectively (Snowdon, 1990) [27]. The natural wax coating on fruits and vegetables is safe to consume. But, the synthetic wax applied on the apples is not easily digested in our body. This unabsorbed wax remains in the small intestine. This wax can cause various problems in the digestive system and cancer. For this development of biological control by using plant extracts is efficacious against the pathogen and it didn't showed any side effects on health whenever we are consuming and no harm to the environment (Zhou *et al*., 2018) [30].

In recent years the use of natural plant extracts in controlling post-harvest diseases and there have been several reported cases of plant extracts having antimicrobial activities (Das *et al*., 2010) [8]. Water loss in apple may also vary significantly among different cultivars resulting in significantly weight loss even under similar storage conditions (Khan and Ahmad, 2005) [18].

Secondary metabolites at very low concentration can express their effects and can easily be adsorbed. Usage of botanical pesticides blend well with strategies for integrated disease management to achieve and ensure that indigenous biodiversity is protected by good crop health and natural enemies. Pesticidal plant research is growing to resolve gaps in our understanding that restrict their adoption (Isman, 2015) [14]. Many plant species with pesticidal properties replace the synthetic fungicides against pathogens that are rapidly growing, and easy to propagate on large scale. They also act as crop fertilizer and assist in increasing yields (Mkindi *et al*., 2017) [23]. The plant compounds also alter the structure of hypha and mycelia, thus inhibiting the development of substances such as aflatoxin from certain fungi, such as *Aspergillus sp*. Toxicity to fungal cell membranes, cell walls and organelles is caused by terpenes, phenols, alkaloids, tannins and other phytochemicals present in botanical pesticides (Kumar *et al*., 2018) [19].

2. Materials and Methods

2.1 Experimental location: The research work was proceeded out at the Department of Plant Biotechnology, University of Agricultural Sciences, GKVK campus, Bengaluru, Karnataka, India. The details of the materials used and methodology are followed under the following headings.

2.2 Culture of *Penicillum expansum*

Penicillium expansum is one of the postharvest disease of apple casing Blue mould disease. *Penicillium expansum* culture was obtained from the Department of Plant Biotechnology, GKVK, Bengaluru, India. The liquid suspension of that culture was made using serial dilution method and spore concentration was made as 10^5-10^6 and counted using Hemocytometer (Ikeura *et al*., 2011) [15].

2.3 Collection of Plant Samples

The fresh and healthy leaves of neem (*Azadirachta indica*), lantana (*Lantana camera*), coleus (*Coleus forskohlii*), lemon grass (*Cymbopogon citratus*) and tulsi (*Ocimum tenuiflorum*) were collected from Botanical Garden, GKVK, Bengaluru. The collected plant samples were brought to Biofuel lab, Department of Biotechnology, GKVK (latitude -13.078353° N,longitude-77.578127° E) After that the plant samples were washed with running tap water in order to get rid of dirt, insects and plankton and kept for shade drying to proceed for further work.

2.4 Preparation of solvent extracts of plant samples using soxhlet apparatus

The various solvent extracts of plants were prepared using soxhlet apparatus based on method followed by (Dhawan *et al*., 2017) [9]. After three to four weeks the shade dried plants were grinded and stored for further work. Solvents selected for extraction was ethanol and water. 25g of powder plant materials used for 250 ml of solvent for extraction and it was extracted using soxhlet apparatus. Once the extraction procedure is completed then the extracted solvent is evaporated using rotary evaporator. Remaining powdered plant extract was taken and stored in small sterilized 5ml screw-capped glass bottles that were refrigerated at 4 °C until further use. Further extracts were diluted with water to get the desired concentrations of 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm respectively.

2.5 *In vitro* screening of plant extracts (Phytochemicals) for antifungal activity by poisoned food technique.

The efficacy of *Lantana camara*, *Tridax procumbens*, *Coleus forskohlii*, *Cymbopogon citratus*, *Azadiracta indica*, *Ocimum tenuiflorum* were evaluated against Post harvest pathogens *Aspergillus niger* and *penicillium digitatum* under *in vitro* conditions on the PDA media using poison food technique (Nene and Thapliyal, 1993) [24]. The experiment was proceeded out using a completely randomized method (CRD). Without adding any plant extracts to the medium, control was retained and each procedure was repeated thrice. Such plates were incubated for seven days at a temperature of 28 ± 2ºC, and radial colony growth (cm) was measured. The efficacy of a plant extract was expressed as percentage inhibition of mycelial growth and that was calculated by using Vincent's formula.

The fungi toxicity of the plant extracts in terms of percent inhibition of mycelial growth was calculated using the formula:

$$I = \frac{C - T}{C} X\ 100$$

Where,
I = Percentage inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

2.6 Experimental design and treatments preparation:

The experiment was based on Completely Randomized Design (CRD) with six treatments and one control and three replications. The six treatments were; T1: Lantana leaf extract, T2: Tridax leaf extract, T3: Coleus leaf extract, T4: Lemon grass leaf extract, T5: Neem leaf extract, T6: Tulsi leaf extract and Control.

2.7 Collection and preparation of fruit samples

Freshly harvested apples of uniform size, good quality and free from any injury or disease were bought from organic farm near university. The fruits were cleaned properly with distilled water to remove all the foreign matters like dust, dirt, mud, filth, etc. Fruits were then grouped in to similar size after washed using sterile distilled water and used for the experiment.

2.8 *In vivo* effect of plant extracts on postharvest quality parameters on fruits

2.8.1 Physiological weight loss (%)

This character was determined in apple with CRD in three replicates for every treatment. The fruits were treated with both solvent extracts (ethanol and aqueous) and control is maintained without any treatment with plant extracts. In each replicate after different storage period using digital electronic

balance and the weight loss percent was calculated by using this formulae (Javaria *et al*., 2012) [16].

Weight loss (%) = ((Initial weight – Final weight) / Initial weight) * 100

2.8.2 Total Soluble Solids (TSS)
For measuring TSS, juice from pulp of randomly selected apple fruits were taken and TSS of the juice was calculated using hand held refractometer of 0-30 percent range. The results obtained were expressed as percentage of total soluble solids of the fruits (Jha *et al*., 2012) [17].

2.8.3 *In vivo* evaluation of disease control ability of plant extracts against *Penicillium expansum* in apple by measuring lesion diameter
Fresh apple fruits were taken and soaked in sodium hypochlorite and washed two to three times with sterile water and kept dry under ambient condition. Each fruit was wounded with sterilized cork borer of 6mm diameter and 5mm depth of total three wounds /fruit. Then 100 micro litre of spore suspension was added to each wound. An hour later that three replicates were subjected to all treatment and one without treatment was kept as control. The treated fruits were kept under ambient temperature of 27 °C and lesion diameter was observed at interval of 4 days upto 12 days. The lesion diameter in fruits were calculated and expressed in centimeter.

2.8.4 Shelf life (Days) of apple treated with plant extracts
Shelf-life of fruits were measured by counting the number of days from start of storage until when more than 50% of samples per replicate have been deteriorated.

2.13. Statistical analysis
The collected data on various parameters were statistically analyzed using OP STAT statistical package program to find out the variation resulting from experimental treatments. Mean comparisons were made using Completely Randomized Design (CRD) test at 1% probability level. All the data were statistically analysed by using analysis of variance (ANOVA) followed by Duncan's Multiple Range test (DMR) at Pd"0.05 (Bashir *et al*., 2019) [3].

3. Results and Discussions
3.1 *In vivo* screening of solvent extracts on *Penicillium expansum* by Poison food technique
The different concentration of ethanolic plant extracts at 3000 ppm, 4000 ppm and 5000 ppm were evaluated against colony growth of *Penicillium expansum.* Among the different plant extracts tulsi extract showed the maximum growth inhibition of 71.11 percent, 53.55 percent and 29.11 percent with colony diameter of 2.6 cm, 4.18 cm and 6.38 cm at all concentrations. Next maximum colony growth inhibition was shown by neem at 5000 ppm with 66 percent inhibition followed by lantana, tridax, lemon grass and coleus in the last with 65.22 percent, 63 percent,
49.11 percent and 44.6 percent. Among aqueous extracts tulsi extract showed maximum inhibition of 41.11 percent with colony diameter of 5.3 cm against the control with 9 cm colony diameter. Next maximum colony inhibition was shown by Neem, lantana, tridax, lemon grass and coleus with 37.55 percent, 33.5 percent, 33 percent, 27.77 percent and 22.44 percent at 5000 ppm concentration.so ethanolic extract of tulsi was good in inhibiting colony growth of *Penicillium expansum*. (Fig. 1)

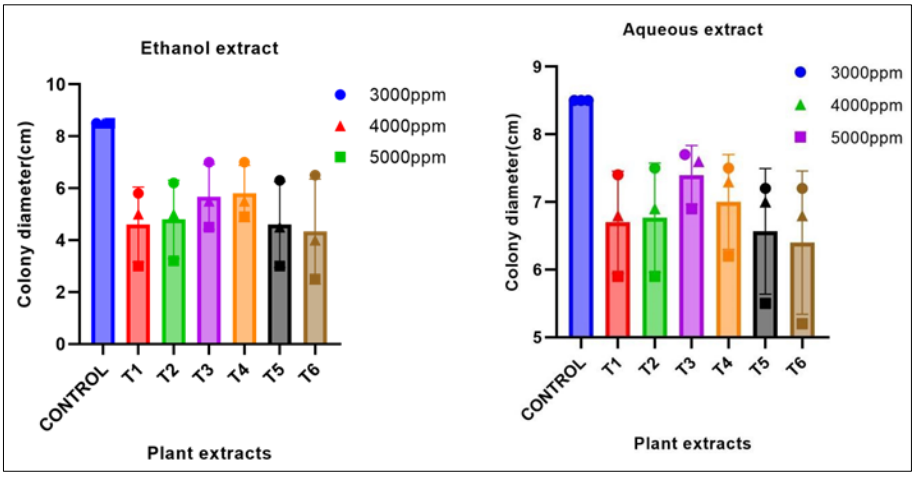

Fig 1: Colony diameter of *Penicillium expansum* treated with plant extracts

3.2 Physiological weight loss (%)
The weight loss in fruits normally depends on the structure and nature of skin and waxes on the surface of the fruit (Babos *et al*., 1984; Veravrbeke *et al*., 2003) [2, 28]. It was assumed that apple weight loss may be responsible for water loss. The physiological loss in weight (PLW) was increased in all the treatments with the advancement of the storage period. The percentage weight loss was calculated in relation to the initial weight of the Apple, which were weighed in frequent intervals for the whole period of 12 days with both ethanol and aqueous plant extracts at different concentration and the results were depicted in the table using statistical analysis (CRD) and the treatment with three replication were maintained and controls were maintained without extracts.
Among aqueous and ethanolic plant extracts maximum weight loss percent was shown by aqueous extract. Ethanolic extract is more effective in controlling weight loss of apple. Among all treatments, maximum weight loss percent was shown by control with 15 percent. In ethanolic extract treated fruits, minimum weight loss percent was shown by tulsi at 5000 ppm (3%) and neem (3.2%) at 5000 ppm respectively. In case of aqueous extract minimum weight loss was observed

in 5000 ppm tulsi extract (8%) followed by neem (8.3%), lantana (8.5%), and maximum weight loss was shown by coleus extract (9.5%). At day 8 minimum weight loss percent was observed in tulsi at 5000 ppm (12%) followed by neem (12.4%), lantana (12.9%), tridax (13.55%), lemon grass (13.5%) and maximum weight loss was shown by coleus (14.5%). At day 12, there was a considerable increase in weight loss percentage in all treatments compared to day 4 and day 8. Among ethanolic extracts, minimum weight loss percent was shown by tulsi (20%) followed by neem (23%), lantana (24%), tridax (27%), lemon grass (29%) and maximum weight loss was shown by coleus (32%) compared to control with 56 percent. In aqueous extract treated fruits minimum weight loss percent was shown by tulsi extract (27%), neem (29%), lantana (32%), tridax (37%), lemon grass (34%) and maximum weight loss was shown by coleus (40%). Minimum weight loss is favourable for increasing the shelf life of fruits. Among the three concentration in both aqueous and ethanol extract minimum weight loss percent was show by 5000 ppm concentration. Among all treatments control is showing maximum weight loss percent. (Fig. 2.). The moisture and subsequent weight loss in fruits were gradually increased with increase in storage duration due to water loss and respiration (Erturk, 2003; Ghafir *et al*., 2009) [11, 13].

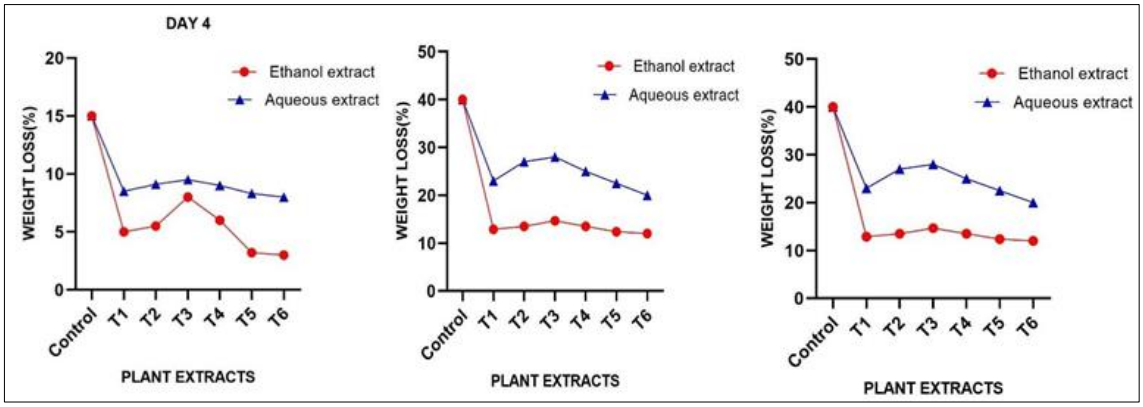

Fig 2: Effect of ethanol plant extracts (5000 ppm) on physiological weight loss (%) of apple during different days until the end of shelf life on ambient room storage (27±2 ºC and 65±5% RH)

3.3 Total soluble solids (TSS)

Statistically significant variation was observed in TSS content of fruits. Total soluble solids of fruits is one of the major qualitative parameter which is mainly related to composition (Peck *et al*., 2006) [25]. The total soluble solids of apples fruit increased gradually with increasing the storage durations. Among aqueous and ethanolic extract maximum TSS was shown by aqueous extract compared to ethanolic extract at day 4. Ali *et al*., (2004) [1] reported significant variations in TSS and other physico-chemical characteristics of apples from the different cultivars under study exhibited non significant variations in total soluble solids (TSS).

TSS of all treatments gradually increased upto storage period of 12 days. Initially there is lower TSS percentage of around 16 in all grapes. The initial increase in TSS may be due to accumulation of sugar as a result of hydrolysis of insoluble polysaccharides (starch) into simple sugars (Shrestha *et al*., 2018) [26]. The increase in TSS of fruits attributed to the breakdown of starch (Beaudry *et al*., 1989) [4] into sugars (Crouch, 2003) or the hydrolysis of cell wall polysaccharides (Ben and Gaweda, 1985) [5]. Among both ethanolic and aqueous extract maximum TSS was found in aqueous extract treated fruits. In 5000 ppm ethanolic extract minimum TSS increase was shown by tulsi extract (15.5) followed by neem (15.9), lantana (15.9). In case of aqueous extract minimum inhibition was shown by tulsi extract at 5000 ppm (16.5) followed by tridax, lantana, lemon grass and coleus. There is slight difference in TSS among both ethanolic and aqueous extract at day 4. At day 8 in both solvent extracts maximum TSS percentage was shown by aqueous extracts. Highest TSS percentage was shown by Control with 18 percent. In case of ethanolic extract minimum TSS increase at 5000 ppm concentration compared to 3000 ppm and 4000 ppm, tulsi extract (16.1) and neem (16.1) is showing minimum increase in TSS compared to other treatment followed by lantana (16.4), tridax (16.5). There is no considerable difference in TSS among 4000 ppm and 5000 ppm. Increase in TSS is associated with less storage life. At day 12, among all treatments tulsi extract (16.7) is showing less increase in TSS percent compared to other plant extract treated fruits. In aqueous extract there is more TSS increase compared to ethanolic extracts. In control there is considerable increase in TSS upto day 8 after that in day 12 there is gradual decrease in TSS. This might be due to decline in organic acid and their consumption for their respiration. Shelf life in inversely related to their respiration. If there is more respiration it will decrease the shelf life of fruits (Fig. 3.).

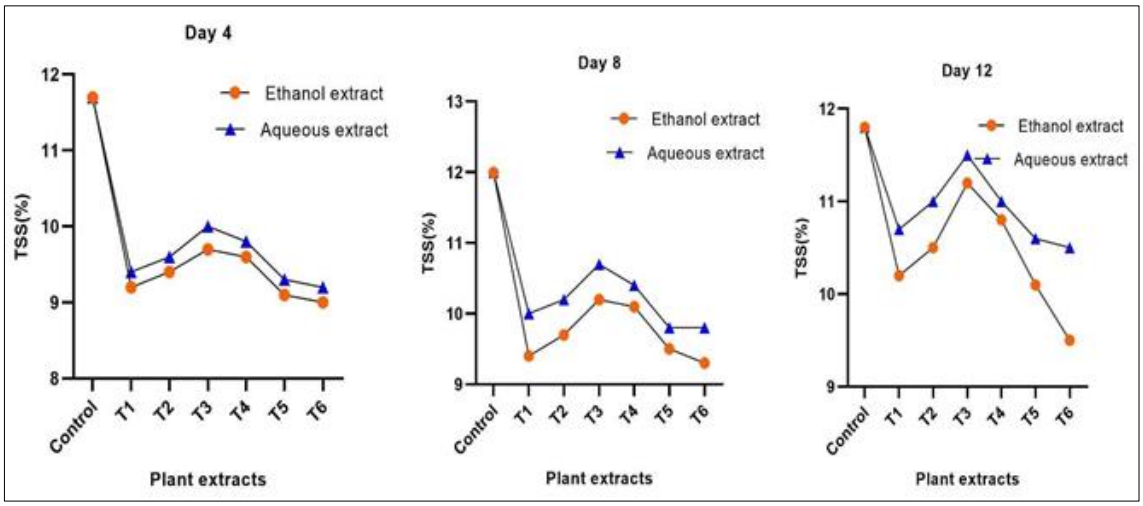

Fig 3: Effect of ethanol plant extracts (5000 ppm) on Total Soluble Solids (%) of apple during different days until the end of shelf life on ambient room storage (27±2 ºC and 65±5% RH)

3.4 Lesion diameter of apple treated with different solvent extracts against *Penicillium expansum*

Lesion diameter of apples inoculated with pathogen *P. expansum* and treated with different plant extracts was observed for 12 days with 4 days interval (CRD). Plant extract which is having more potential in inhibiting the mycelial growth in fruits *in vivo* was having antifungal activity against this pathogen.

Ethanolic extract of plants showed more antifungal activity in controlling *P. expansum* compared to aqueous plant extracts. Among ethanolic solvent extracts minimum lesion diameter was shown by tulsi (2.5 cm), neem (2.7 cm), followed by lantana (3cm) at 5000 ppm at day 12. Maximum lesion diameter was shown by control with 6 cm diameter. Among different plant extracts maximum lesion diameter was shown by fruits treated with coleus (4 cm). It is showing less antifungal activity against *P. expansum*. In case of aqueous extracts minimum lesion diameter was shown by tulsi, neem and lantana with lesion diameter of 3.5 cm, 3.7 cm and 3.9 cm respectively. Maximum lesion diameter was shown by control with 6 cm diameter. Comparing both ethanolic and aqueous extracts, ethanolic extract is showing maximum antifungal activity against *P. expansum* (Fig. 4.)

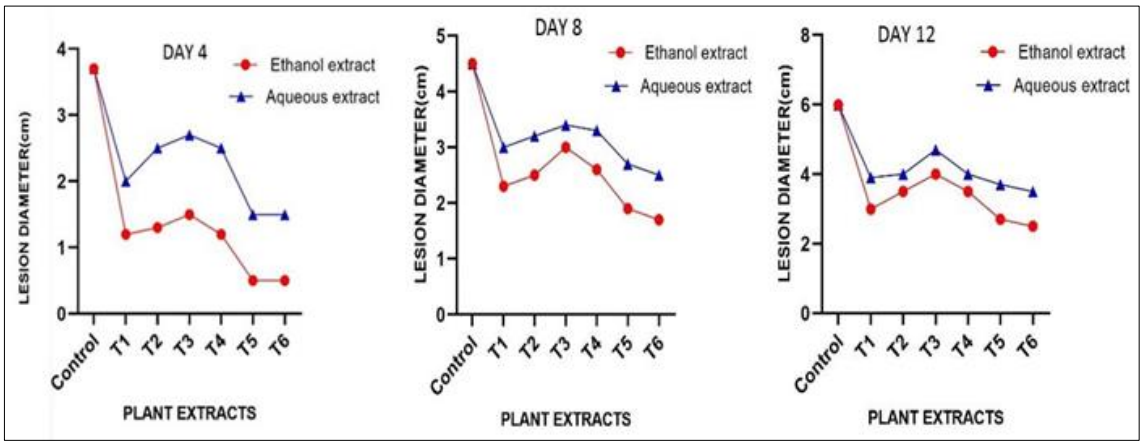

Fig 4: Effect of ethanol and aqueous plant extracts on lesion diameter of apple during different days until the end of shelf life on ambient room storage (27±2ºC and 65±5% RH)

3.5 Shelf life of Apples treated with plant extracts

Apple shelf life was increased when treated with ethanolic plant extracts at concentration of 5000 ppm. Beyond this concentration (5000 ppm) there was a considerable colour change in fruits was observed. Among all plant extracts treated with fruits ethanolic extract of tulsi, neem and lantana at 5000 ppm concentration was showing good results with shelf life of 14 days compared to control with shelf life of only 9 days. In case of aqueous extract treated apple fruits the maximum shelf life was upto 10 days (Tulsi and neem @ 5000 ppm). Among ethanolic and aqueous extracts aqueous extract gave best result in case of apple with increased shelf life of fruits. The disease infestation was also lower in tulsi, neem and lantana ethanol leaf extracts treated fruits and that fruits appearance is also good (Fig. 5.). Phytochemicals present in the plants may be responsible for the antimicrobial activity (Mani *et al*., 2017) [2].

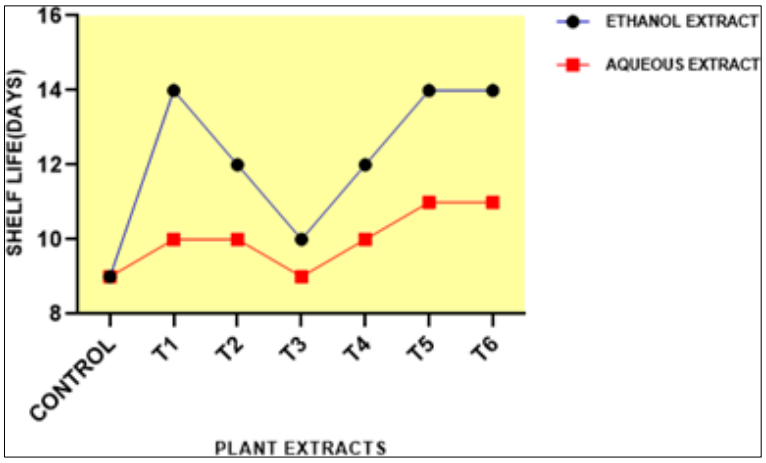

Fig 5: Shelf life of fruits sprayed with different plant extracts in apple

4. Conclusion

In this present investigation six plant extracts namely Lantana, Tridax, Coleus, Lemon grass, Neem and Tulsi were tested as an antifungal agent against *Penicillium expansum* which cause major post-harvest loss in apple. Six plant extracts were found to be effective in controlling *P. expansum* in apple. Among six plant extracts ethanolic plant extracts were effective compared to aqueous plant extracts as an antifungal agent. Using poisoned food technique, percentage of mycelial growth inhibition was calculated. Among ethanolic & aqueous plant extracts maximum rate of mycelial inhibition was shown by ethanolic of extract tulsi (71.11%) followed by neem (66%) respectively. Shelf life of fruits were evaluated by checking the parameters like weight loss percentage, Total soluble solids (Brix%) in apple. Minimum weight loss percent was shown by ethanolic extract of tulsi (20%), neem (23%) and lantana extract (24%) compared to control (56%). Minimum TSS percent was shown by ethanolic extract of tulsi (9.5%), neem (10.1%) and lantana (10.2%) compared to control with 11.8 percent. In control there was considerable increase in TSS upto day 8 after that TSS% decreases which may be due to the decline in organic acid & their consumption for respiration. Shelf life is inversely proportional to respiration. Shelf life of fruits were observed by noticing the fruit quality, colour and other visible parameters. In apple Tulsi, Neem & lantana ethanolic extract at 5000 ppm is showed maximum shelf life of 14 days. It may be due to phytochemicals present in plants that is having antimicrobial activity. Our results also showed that the inhibitory role of bioactive phytochemicals from ethanolic extract of *Occimum tenuiflorum* against *Penicillium expansum.*

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7. Conflict of interest

The authors declare that they have no conflict of interest in the content of the manuscript and study undertaken. The article is original and has not been submitted elsewhere for publication.

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