Research Article

Postharvest Management of Green Mold of Citrus with Medicinal and Aromatic Plants

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Abstract

The efficacy of water extracts and essential oils of five locally available medicinal and aromatic plants viz. ginger (*Zingiber officinale* Rosc), garlic (*Allium sativum* L.), lemongrass (*Cymbopogon flexuosus* Stud), Japanese mint (Mentha arvensis L.) and holy basil (*Ocimum sanctum* Linn.) were in vitro evaluated against the fungus *Penicillium digitatum* Sacc. and the green mold disease of *citrus* fruits (in vivo) at central laboratory of IAAS, Rampur, Chitwan during the winter season of 2007. This experiment was conducted in completely randomized design with five replications. Extracts were either tested alone, or in combination with vegetable (mustard) cooking oil or bee wax at different concentrations using mandarin fruit cultivar (local Suntala). Treated fruits were stored at 15 ± 1ºC and 90-95 % relative humidity for 15 days. Further experimentation was carried out by lowering the concentration levels of the extract. All the extracts of various concentrations were effective as compared to control in inhibiting the growth and development of *Penicillium digitatum* Sacc. However the most effective one was lemongrass oil in the management of green mold disease of citrus. A markedly increased ability of extracts was observed when extracts were used with bee wax in checking green mold disease of citrus fruits. The treatment comprising lemongrass oil and mint oil even at lower concentration level (0.01-0.04%) was also found equally effective as that of the fungicide treatment in controlling green mold on mandarin oranges.

Key words: Medicinal and aromatic plants; green mold; post-harvest management; *Penicillium digitatum* Sacc

Introduction

The productivity of most important and highly commercialized fruit crop-citrus (mainly mandarin orange) in Nepal is reducing due to several factors. Among them pests and disease are the major ones. In recent years, the blue and green molds (*Penicillium* species) have become increasingly important on post-harvest losses of citrus in Nepal (Gautam *et al*., 2002). The citrus fruits mainly mandarin, as any other fruits, are perishable in nature. The postharvest glut situation during the harvesting season (mid-November to mid-January) results in low prices and marketing problems, resulting in considerable losses to farmers. This situation has endangered the entire Citrus enterprise in the country resulting in a negative impact on the livelihoods of mid hill farmers (Paudel *et al*., 2004). Encouraging results on the use of natural products to control postharvest fungal rotting indicate that we should be able to develop natural fungicides that would be as effective as synthetic fungicides, and presumably safer for man and the environment. Chemical fungicides provide the primary means for controlling postharvest fungal decay of fruit and vegetables. Continuous use of fungicides has faced two major obstacles—increasing public concern regarding contamination of perishables with fungicidal residues, and proliferation of resistance in the pathogen populations. The ultimate aim of this research in this area has been the development and evaluation of various alternative control strategies to reduce dependency on synthetic fungicides. Several non-chemical treatments under in vitro and in vivo analysis have been proposed for fungal decay control.

Materials and Methods

The mandarin fruits local cultivar having similar maturity stages (75-80%) were carefully picked with short stems using clipping knife from a private farm of Gorkha district. Matured orange fruits having yellowish color were selected and packed in poly bags and kept in plastic crates without any post-harvest treatment and were transported to Rampur (100 km). Highly aggressive isolate of *Penicillium digitatum* Sacc originally isolated from citrus fruits was taken. The isolate was maintained on potato dextrose agar (PDA) at the central lab (IAAS, Rampur, Department of Plant Pathology). Some plates with the pathogen was maintained in the lab for judging the efficacy of botanical extracts by poisoned food technique and the rest plates were used for extracting the spores for artificial inoculation to the mandarin fruits. Spores (Conidia) were harvested by flooding the media with sterile distilled water and gently scraped from 7 day old culture. It was filtered through muslin cloth to remove the conidiophore and mycelium. The conidial suspension of 10^6 spores per ml concentration was maintained with the help of haemocytometer.

The medicinal and aromatic plants viz; ginger (*Zingiber officinale* Rosc) (local cultivar), garlic (*Allium sativum* L.) (Local cultivar), lemongrass (*Cymbopogon flexuosus* Stud), Japanese mint (*Mentha arvensis* L.) and holy basil or tulsi plant (*Ocimum sanctum* Linn.) (Local cultivar) used in the experiment were growing in the organic farm of one hectare at the town Bhagar area of Nawalparasi district. These plants were cultivated by the ethnic groups of this particular area with the technical contribution of French Ingo Human Care through participatory approach.

The garlic and ginger extracts were prepared by taking fresh samples. While preparing the extracts, the outer, dry peel of cloves was first removed, surface-sterilized for 2 min in 70% ethanol, and washed in three changes of sterile distilled water. Hundred gram cloves of both plants were weighed separately and grinded into the pulp separately using an electrical grinder. The pulp was squeezed and filtered through sterile cotton wool in to a 200 ml conical flask. The volume of the filtrate collected was 50 ml and made up to 100 ml with the addition of 50 ml sterile distilled water (Obagwu and Korsten, 2003). The Japanese mint and Basil plant extracts were prepared from fresh samples. In preparing the extracts, unwanted and hard stem were first removed and just selected the succulent leaf parts, surface-sterilized for 2 min in 70% ethanol, and washed in three changes of sterile distilled water. Hundred gram fresh leaves of both plants were weighed and grinded into the paste separately using an electrical grinder. The paste was squeezed and filtered through sterile cotton wool into a 200 ml conical flask. The volume of the filtrate collected was 50 ml and made up to 100 ml with the addition of 50 ml sterile distilled water.

Lemongrass oil used in this experiment was received from distillation plant of Nawalparasi district (Community Herbal Producers Cooperative Society Limited, Gaindakot). The distillation plant is of stainless steel and produces rust free oil. The color of oil used in the experiments was yellow. Mint oil used in this experiment was also received from distillation plant of Nawalparasi district (Community Herbal Producers Cooperative Society Limited, Gaindakot area). The color of oil used in the experiment was yellowish white.

Honey bee wax used in this experiment was received from Sagar Bee Hive Industry and Resource Center, Gaindakot-8. The species of honey bee was *Apis melifera* L. and the color of wax was yellowish white.Mustard (*Brassica camprestris* L. var. *dichotoma* (Roxb.) Kitam) oil used in this experiment was received from Pragati oil industry, Gaindakot-8 and the color of oil used in this experiment was yellow.

Potato dextrose agar was prepared in the central laboratory of Rampur agricultural college. Two hundred gram of washed, peeled and chopped potato tubers were boiled in 1 liter of distilled water till the potato partially cooked. After filtration of potato extract through muslin cloth, 20 gm dextrose and 20 gm agar were added and sterilized in autoclave at 15 psi for 15 minutes at 121^{o}C.

In-vitro test

In-vitro test of medicinal and aromatic plant extracts against *P. digitatum* Sacc was done at the central laboratory, Department of plant pathology, at room temperature (14-16 ^{o}C) for 13 days. The experiment was carried out by poisoned food technique. Different concentration of water extracts of organic medicinal and aromatic plants local cultivar were incorporated into PDA individually in different conical flasks, each containing 100 ml of sterile molten PDA. The extracts were added to the PDA when it is cooled to about 50 ^{o}C with the help of micro-pipette. The flasks were gently agitated for 2 minutes to allow for a proper mixing of extract with the nutritive medium. Then it was poured in to the Petri-plates (9cm diameter) @ 20 ml / plate. Dicrysticin-S (250 mg per liter) was added to the medium at the time of pouring to prevent bacterial contamination. There were altogether six different treatments consisting of garlic, ginger, holy basil, Japanese mint, lemongrass extracts and control. The extracts of each medicinal and aromatic plant were further diluted by adding different amounts in the same volume of PDA so as to make the concentration levels 0.05%, 0.25%, 0.5% and 0.75% while in case of lemongrass extract the concentration was maintained to 0.1%, 0.5%, 1%, and 1.5%. Five plates were considered as one experimental unit and replicated 5 times. Each experiment was carried out in completely randomized design (CRD). Further experiments were carried out using rather lower concentration levels of the medicinal and aromatic plant extracts to confirm their efficacy in checking the growth of pathogen. There were 3 experimental setup consisting of Garlic extract, lemongrass oil and mint oil. Each experiment included 5 treatments; garlic extract at 0.005%, 0.01%, 0.015% and 0.02% concentration levels while lemongrass oil and mint oil the concentration used were 0.01%, 0.02%, 0.03% and 0.04% including control (without any extract). Five plates were considered as one experimental unit and replicated 5 times. The experiments were conducted in completely randomized design.

Four millimeter diameter of *P. digitatum* Sacc of one week old culture was cut by cork borer and picked up with the help of inoculating needle and placed on to the centre of the plate. The plate contained PDA amended with medicinal and aromatic plant extract and the cut piece was kept upside down for better contact of pathogen to the media. The plates were incubated at room temperature (14-16 ^{o}C) for up to 13 days. The colony diameter (cm) of the pathogen was determined by measuring the average radial growth on 3, 5, 7, 9, 11, and 13th day of incubation. Average radial growth was recorded by using a measuring scale from the lower view of the petri-plates. Room temperature was also

recorded every day during the experimental period. Two sets of experiments were carried out in the lab and the second set of experiment comprised the plant extract selected on the basis of their performance in the first experiment. The first observation was recorded on 9th December 2007 for the first experiment setup and the setup contained the effect of plant extracts such as; garlic extract, ginger extract, holy basil extract, Japanese mint extract and lemongrass oil extracts on pathogen growth while in case of second experiment, the first record was taken on 15th January 2008 and it contained the effect of plant extracts such as; garlic extract, lemongrass oil and mint oil on pathogen growth.

In-vivo test

In-vivo test was done at Central Laboratory, IAAS, Rampur, Chitwan, Nepal. The experiments were conducted in an environmental growth chamber. Fresh, healthy fruits of similar maturity were surface-sterilized with 70% ethanol for 1–2 min, and fruits were dipped in medicinal or aromatic plant extracts prepared as described above directly and further they were treated by mustard oil or bee wax. Honey bee wax was kept in the steel vessel and melted in heater and the mandarin oranges were dipped in the melted wax. The run-off collected in sterile containers was re-used. Twenty four hours after dipping in the above treatments, the fruits were wound-inoculated with *P. digitatum* Sacc. *(*1×10^6 spores per ml), by pricking, using sterile injection syringe. One wound, each approximately 1mm wide and 5mm deep, was made at the stem end of each fruit. Treated fruits were stored in paper boxes (good quality hard paper) at room temperature (15^0C), and 90–95 % Relative Humidity (RH) for 15 days, and assessed thereafter for decay symptoms. There were altogether 16 treatments; ginger extract (T_1), ginger extract + mustard cooking oil (T_2), ginger extract + honey bee wax (T_3), mint extract (T_4), mint extract + mustard cooking oil (T_5),mint extract + honey bee wax (T_6), holy basil (Tulsi) extract (T_7), holy basil extract + mustard cooking oil (T_8), holy basil extract + honey bee wax (T_9), lemongrass oil (T_{10}), lemongrass oil + mustard cooking oil (T_{11}), lemongrass oil + honey bee wax (T_{12}), garlic extract (T_{13}), garlic extract + mustard cooking oil (T_{14}), garlic extract + honey bee wax (T_{15}) and Control (T_{16}). Five fruits were taken as single experimental unit and each treatment was replicated five times in completely randomized design. The control consisted of fruits immersed in sterile distilled water.

Further experiment was carried out with different treatments based on the performance of the first experiment to confirm the effect of medicinal and aromatic plant extracts on the disease control. There were altogether 8 treatments that included mature fruit dipped in mint oil + honey bee wax (T_1), garlic extract + honey bee wax (T_2), lemongrass oil + honey bee wax (T_3), honey bee wax (T_4), lemongrass oil (T_5), garlic extract (T_6), mint oil (T_7)and control (T_8). A single treatment contained 5 fruits and each treatment was replicated four times in completely randomized design. The control consisted of fruits immersed in sterile distilled water.

Disease assessment was done in 0-5 scale. This assessment was based in the rotted area with respect to total surface area of the orange viewed by naked eyes and expressed in percentage.

0 = no infection (healthy fruits)

1 = infection start (0-5% rotting)

2 = 6-10 % rotting

3 = 11-15 % rotting

4 = 16-20% rotting

5 = > 20% rotting.

The observation was based on the scale developed by Obagwu and Korsten, 2003.The observations were recorded from 26th December 2007 for the first experiment while for second experiment; observations were recorded from 1st February 2008 to find out the effect of extracts on disease control.

Disease checked was calculated by the following formula:

$$\text{Percent disease checked} = \frac{\text{Disease intensity of control} - \text{Disease intensity of treatment}}{\text{Disease intensity of control}} \times 100$$

Data were statistically analyzed using MSTAT-C software by analysis of variance and the significance of the treatments were determined by using Least Significance Difference (LSD) and Duncan's multiple range test (DMRT). Descriptive analysis was also conducted. Ms-Excel package was used for data entry.

Results

In vitro test

Comparative efficacy of different plant extracts

The comparative efficacy of different medicinal and aromatic plant extract incorporated PDA on the growth of *P. digitatum* Sacc. on 13th day of incubation period at room temperature (14.5-15.5 ^{0}C) is presented in Fig. 1. The lemongrass oil amended PDA had highly significant ($p<0.05$) effect on checking the growth of the *P. digitatum* Sacc.as compared to control. There was no further growth of the pathogen on the PDA incorporated with lemongrass oil irrespective of the concentrations used and number of days incubated. Besides lemongrass oil, other aromatic and medicinal plant extract could not completely check the growth of *P. digitatum* Sacc. However, they were able to reduce the rate of growth of the pathogen as compare to

control. The garlic extract was most effective followed by mint extract, ginger extract and basil extract in reducing the growth of *P. digitatum* Sacc. The aromatic and medicinal plant extract incorporated PDA had significant ($p<0.05$) effect on the growth of the *P. digitatum* Sacc. at room temperature (14.5-15.5 ^{0}C) as compared to control in all durations of growth except in ginger and basil extract up to 9 days of incubation period (Table 1). The growth of *P. digitatum* Sacc on PDA was 8.61 cm in diameter when incubated for 13 days while it was only 0.4 cm, 6.01 cm, 6.36 cm, 6.90 cm and 7.32 cm in diameter when lemongrass oil, garlic extract, mint extract, ginger extract and basil extract respectively were incorporated in to the PDA.

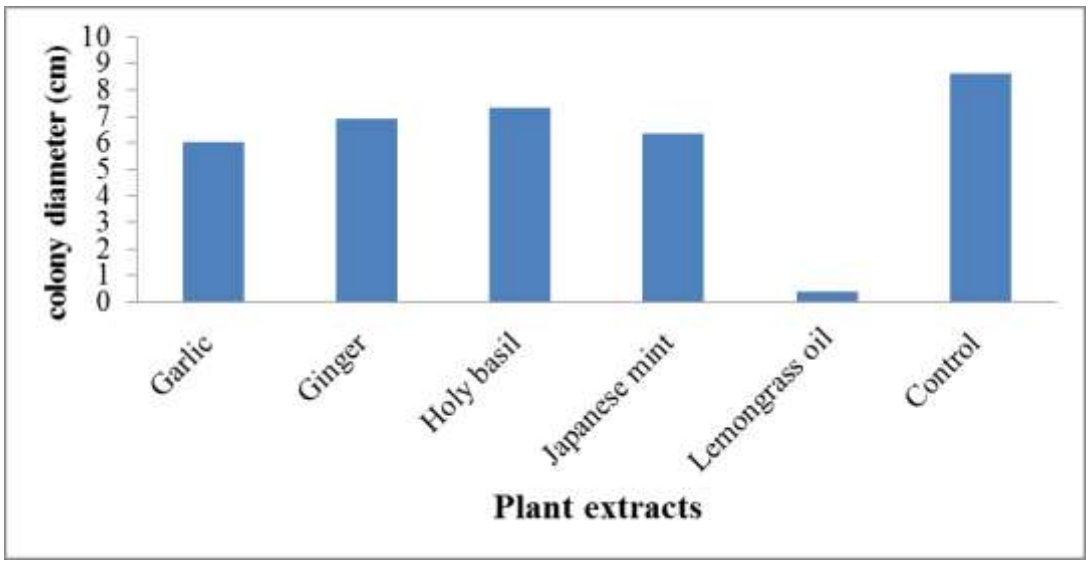

Fig.1: Effect of different medicinal and aromatic plant extract incorporated PDA on the growth of *P. Digitatum* Sacc. on 13th day of incubation period at room temperature (14.5-15.5 ^{o}C)

Table 1: Effect of different medicinal and aromatic plant extract incorporated PDA on the growth of *P. Digitatum* Sacc. in different incubation period at room temperature (14.5-15.5 ^{o}C)

Treatments	**Mean colony diameter (cm)**					
Plant extracts	**3rd day**	**5th day**	**7th day**	**9th day**	**11th day**	**13th day**
Garlic	0.87 ^{c†}	2.06 ^{b}	2.83 ^{b}	3.79 ^{c}	4.83 ^{d}	6.01 ^{e}
Ginger	1.28^{ab}	2.21^{ab}	3.24 ^{a}	4.40 ^{ab}	6.03 ^{b}	6.90 ^{c}
Holy basil	1.35 ^{a}	2.42 ^{a}	3.24 ^{a}	4.61 ^{a}	6.03 ^{b}	7.32 ^{b}
Japanese mint	0.91 ^{c}	1.98 ^{b}	3.22 ^{a}	3.86 ^{c}	5.48 ^{c}	6.36 ^{d}
Lemongrass oil	0.40 ^{d}	0.40 ^{c}	0.40 ^{c}	0.40 ^{c}	0.40 ^{e}	0.40 ^{f}
Control	1.16 ^{b}	2.49 ^{a}	3.48 ^{a}	4.27 ^{b}	6.42 ^{a}	8.61 ^{a}
F- value	67.15	66.03	165.23	269.38	862.58	6632.23
LSD (≤0.05)	0.12	0.27	0.26	0.28	0.22	0.10
CV %	9.57	11.04	7.39	6.05	3.53	1.32
SEm±	0.04	0.09	0.09	0.09	0.07	0.03

†Means of 5 replication. Means in column with same superscript is not significantly different by DMRT $_{(P<0.05)}$ PDA = Potato Dextrose Agar

In vivo test

Effect of medicinal and aromatic plant extracts

All the medicinal and aromatic plant extracts used alone or in combination with either mustard oil or honey bee wax were found significantly effective in controlling the green mold disease of mandarin orange in the storage except ginger extract used alone (Table 2). The disease scores on the control sets were 0.84, 3.52 and 5.00 after 3, 9 and 15 days of storage at 15-18 ^{0}C, respectively, where as in case of lemongrass oil treated sets the disease scores were 0.00, 0.00 and 0.32 respectively. In all the medicinal and aromatic plant extracts, the efficacy of disease control in the storage of mandarin orange was enhanced when used in combination with vegetable oil or bee wax. Among the treatments, lemongrass oil was found the most effective followed by garlic extract, mint extract, holy basil extract and the least by ginger extract. The green mold disease of mandarin orange was completely checked up to 3 days by lemongrass oil, garlic extract and mint extract (Table 3). The disease was completely checked up to 9 days of storage with lemongrass oil. The ginger extract and holy basil extract could significantly control only up to 7.20% and 4.80% disease where as in case of lemongrass oil, garlic extract and mint extract the disease was controlled up to 93.60%, 63.20% and 42.40%, respectively after 15 days of storage. The disease control was significantly enhanced further up to 98.40%, 78.40% and 52.80%, respectively when used in combination with honey bee wax.

Table 2: Effect of different medicinal and aromatic plant extract alone or in combination with mustard oil or honey bee wax against citrus green mold in the storage at 15 -18 ^{o}C

Treatments	**Mean disease intensity (%)**		
	After 3 day	**9 day**	**15 day**
Ginger extract	0.56 ^{abc†} (1.02)	2.60 ^{ab} (1.71)	4.64 ^{ab} (2.27)
Ginger + Mustard oil	0.40 ^{bcd} (0.93)	2.16 ^{bc} (1.59)	4.20 ^{b} (2.17)
Ginger + Bee wax	0.28 ^{cd} (0.87)	1.32 ^{cde} (1.31)	3.12 ^{c} (1.89)
Mint extract	0.00 ^{e} (0.71)	0.68 ^{ef} (1.08)	2.88 ^{cd} (1.83)
Mint + Mustard oil	0.00 ^{e} (0.71)	1.08 ^{de} (1.25)	3.04 ^{c} (1.88)
Mint + Bee wax	0.00 ^{e} (0.71)	0.40 ^{fg} (0.94)	2.36 ^{de} (1.69)
Holy basil extract	0.64 ^{ab} (1.07)	2.56 ^{ab} (1.75)	4.76 ^{ab} (2.29)
Basil + Mustard oil	0.28 ^{cd} (0.87)	1.88 ^{bcd} (1.54)	4.60 ^{ab} (2.26)
Basil + Bee wax	0.16 ^{de} (0.80)	1.28 ^{cde} (1.31)	3.12 ^{c} (1.89)
Lemongrass oil	0.00 ^{e} (0.71)	0.00 ^{g} (0.71)	0.32 ^{h} (0.90)
Lemongrass oil + Mustard oil	0.00 ^{e} (0.71)	0.16 ^{fg} (0.81)	0.88 ^{g} (1.17)
Lemongrass oil + Bee wax	0.00 ^{e} (0.71)	0.00 ^{g} (0.71)	0.08 ^{h} (0.76)
Garlic extract	0.00 ^{e} (0.71)	0.32 ^{fg} (0.90)	1.84 ^{f} (1.52)

Treatments	Mean disease intensity (%)		
	After 3 day	9 day	15 day
Garlic + Mustard oil	0.00 [e] (0.71)	0.60 [ef] (1.05)	2.24 [ef] (1.65)
Garlic + Bee wax	0.00 [e] (0.71)	0.20 [fg] (0.83)	1.08 [g] (1.25)
Control (distilled water)	0.84 [a] (1.14)	3.52 [a] (1.99)	5.00 [a] (2.34)
F-value	8.40	17.32	78.02
LSD value(≤0.05)	0.14	0.27	0.16
C.V. %	14.06	17.79	7.31
SEm±	0.05	0.10	0.06

[†]Means of 5 replication. Means in column with same superscript is not significantly different by DMRT $_{(P<0.05)}$ .Mean in parenthesis is of transformed data

Table 3: Mean percentage disease checked of *P. digitatum* Sacc.in mandarin orange with respect to different medicinal and aromatic plant extract

Treatments	Percentage disease checked in storage		
	3 day	9 day	15 day
Ginger extract	33.33 [cd]	26.13 [d]	7.20 [fg]
Ginger + Mustard oil	52.38 [bc]	38.63 [d]	16.00 [f]
Ginger + Bee wax	66.66 [b]	62.50 [c]	37.60 [e]
Mint extract	100.00 [a]	80.68 [bc]	42.40 [de]
Mint + Mustard oil	100.00 [a]	69.31 [c]	39.20 [e]
Mint + Bee wax	100.00 [a]	88.63 [abc]	52.80 [cd]
Holy basil extract	23.80 [d]	27.27 [d]	4.80 [fg]
Basil + Mustard oil	66.66 [b]	46.59 [d]	8.00 [fg]
Basil + Bee wax	80.95 [ab]	63.63 [c]	37.60 [e]
Lemongrass oil	100.00 [a]	100.00 [a]	93.60 [a]
Lemongrass oil + Mustard oil	100.00 [a]	95.45 [ab]	82.40 [b]
Lemongrass oil + Bee wax	100.00 [a]	100.00 [a]	98.40 [a]
Garlic extract	100.00 [a]	90.90 [ab]	63.20 [c]
Garlic + Mustard oil	100.00 [a]	82.95 [abc]	55.20 [c]
Garlic + Bee wax	100.00 [a]	94.31 [ab]	78.40 [b]
Control	0.00 [e]	0.00 [e]	0.00 [g]
F-value	14.49	17.93	67.50
LSD value(≤0.05)	22.60	19.90	11.11
C.V. %	22.53	23.77	19.63
SEm±	7.99	7.03	3.93

[†]Means of 5 replication. Means in column with same superscript is not significantly different by DMRT $_{(P<0.05)}$

Discussion

The experiments conducted to see the efficacy of different medicinal and aromatic plant extracts on controlling the green mold disease of mandarin orange in the storage at different time interval clearly indicated that there is significant effect of these extracts as compared to control (without any treatment) in controlling the disease.In vitro test of these plant extracts showed better checking of *P. digitatum* Sacc. growth with garlic and mint extract as compared to holy basil and ginger extract at 1.5 % on PDA. The results also showed that different levels of concentration had significantly different effect on the growth of the pathogen i.e. increase in concentration level of extract results decrease in growth of the pathogen. This finding agrees with earlier reports that water and ethanol extracts of garlic cloves at all concentrations checked the growth of artificially inoculated *P. digitatum* and *P. itallicum*, the cause of citrus green and blue mold respectively, as compare to water control (Obagwu and Korsten, 2003). The media incorporated with lemongrass oil completely inhibited the growth of *P. digitatum* Sacc irrespective of the concentrations used. This result is in agreement with the report of Abd-El-Khair *et al*. (2006) who found lemongrass extract could inhibit the mycelial growth of pathogenic fungus *P. digitatum*. Inouye *et al*. (2000) also found fungicidal activity in lemongrass essential oil followed by cinnamon bark and thyme oils, by vapor contact.

Although all concentrations of individual extracts were significantly better in controlling the disease as compared to control, they were not as effective as the lemongrass oil treatments, which gave 100 % control under in vivo test. The results showed that the best control was achieved with lemongrass oil + bee wax treatment followed by garlic extract + bee wax treatment. The results also showed that good control of the pathogen was achieved with garlic extract treatments. This finding is in agreement with the findings of Singh *et al.*, (1993), Shimoni *et al*., (1993), Adegoke&Odesola (1996), Daferera *et al*.,(2000) and Obagwu&Korsten, (2003). They reported that the crude extracts of medicinal plants, i.e. lemon grass and garlic gave good antifungal activity against fungal diseases development under stored conditions. The antifungal activity of medicinal plant extract may be due to chemical constituents of plants such as phenolic compounds found in garlic. A significant increase in the biological activity of extracts was observed when treatments were combined with bee wax. The treatments comprising ginger, mint, basil, garlic and lemongrass oil combined with bee wax showed very good result in controlling the green mold of mandarin orange.

Conclusion

The extracts of garlic, ginger, holy basil, mint and lemongrass essential oil were evaluated alone or in combination with mustard oil and bee wax. Extracts were more effective in inhibiting green mold of citrus caused by *P. digitatum* Sacc. A remarkable improvement in biological activity was observed when extracts were combined with bee wax in the citrus. As a result, treatment comprising lemongrass essential oil + bee wax was as effective as fungicides in controlling green mold of citrus.

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