ISSN 0974 – 5211

Journal of Natural Products

Volume 6 (2013)

www.JournalofNaturalProducts.Com

Research Paper

Pesticidal activity of *Ginger* oil against post harvest spoilage in *Malus pumilo* L.

Sushil Kumar Shahi[1*], Mamta Patra Shahi[2]

[1]Bioresource Tech Laboratory, Microbiology Department, CCS University, Meerut- 250005, India

[2]Microbiology Department, Meerut Institute of Engineering and Technology, Meerut-250005, India

* Corresponding Author

(Received 18 July 2011; Revised 21 July 2011-23 December 2012; Accepted 28 December 2012)

ABSTRACT

In vitro Ginger oil *(Zingiber officinale* Rosc.*)* showed potent bioactivity against dominant post harvest fungal pathogens. The minimum bioactive concentrations (MBCs) with fungicidal action of the oil was found to be 1.0µl/ml for *Alternaria alternata, Botrytis cinerea, Cladosporium cladosporioides, Colletotrichum capsici, C. falcatum, Fusarium cerealis, F. culmorum, Gloeosporium fructigenum, Penicillium digitatum, Penicillium expansum, P. italicum, P. implicatum, P. minio-luteum,* 1.2µl/ml for *Aspergillus flavus, A. fumigatus, A. niger, A. parasiticus, Curvularia lunata, Fusarium oxysporum, F. udum, Penicillium variable, Helminthosporium oryzae, H. maydis, Phoma violacea,* and 1.4µl/ml for *Rhizopus nigricans*. The oil exhibited potency against heavy doses (30 mycelial agar disc, each of 5mm in diameter) of inoculum at 2.0µl/ml concentrations. The bioactivity of the oil was thermostable up to 100°C and lasted up to 48 months. The oil preparation did not exhibit any phytotoxic effect on the fruit skin (epicarp) of *Malus pumilo* up to 50µl/ml concentrations. *In vivo* trials of the oil as a fungicidal spray on *Malus pumilo* for checking the rotting of fruits, it showed that 30µl/ml concentration controls 100% infection by pre-inoculation treatment, while in post-inoculation treatment, 40µl/ml concentration of fungicidal spray were required for the 100% control of rotting. The fungicidal spray was found to be cost effective (INR-10/L) has long shelf life (48-month) and devoid of any adverse effects. Therefore, it can be used as a potential source of sustainable eco- friendly broad-spectrum herbal pesticide after successful completion of wide range trials.

Keywords: *Zingiber officinale;* Fungicidal spray; Fruit rot; Herbal pesticide; *Malus pumilo.*

INTRODUCTION

Edible fruits are among the most important foods of mankind as they are nutritive and indispensable for the maintenance of health. They are also high-value commodities, offering good economic return even on small area of land. India, being geographically subtropical country with warm and humid climate, provides suitable environment for developing and spread of numerous plant pathogens. Harvested fruit and vegetables are attacked by microorganisms because of their high moisture content and rich nutrients (Simmonds, 1963). Usually synthetic pesticides are applied for the control of 'pest and disease' of the agricultural food commodities, as these are effective,

dependable and economic. However, there indiscriminate use has resulted into several problems such as pest resistance to pesticides, resurgence of pests, toxic residues in food (causing health hazards to animals and human beings), water, air, soil and disruption of eco-system (Somasundaram, et al., 1990).

Natural products are an alternative to the use of these synthetic pesticides (Shahi, et al., 2003). Keeping this view in mind, the present paper reports the bioactivity of the essential oil of *Zingiber officinale.* It is a plant of very ancient civilization and the species has long been used in Asia. It is one of the earliest known spices in Europe and is still in large demand.

In the present investigation the oil of *Zingiber officinale* Rosc were evaluated *in vitro* against dominant post harvest pathogenic fungi as well as control of rotting in apple (*Malus pumilo* L.).

MATERIALS AND METHODS

Maintenance of fungus culture: The test fungal pathogens, *Alternaria alternata* (Fr.) Keissler (MTCC 2724), *Aspergillus flavus* Link (MTCC 3396), *A. fumigatus* Fres (MTCC 2544), *A. niger* Van Tiegham (MTCC 1781), *A. parasiticus* Speare (MTCC 6768), *Botrytis cinerea* Pers. Ex. Fr. *(*MTCC 2104), *Cladosporium cladosporioides* (Fresenius) de Vries (MTCC 3478), *Colletotrichum capsici* (Syd.) Butler and Bisby (MTCC 2071), *C. falcatum* Went. (MTCC 2222), *Curvularia lunata* (Wakker) Boedijn (CBTC 2342), *Fusarium cerealis* (Cooke) Sacc. (CBTC 2456), *F. culmorum* (W.G Smith) Sacc. (MTCC 2090), *F. oxisporum* Schlecht.:Fr. (MTCC 2087), *F. udum* (Butler) Snyder and Hansen (MTCC 2204), *Gloeosporium fructigenum* Berk. (MTCC 2191), *Helminthosporium oryzae* Breda de Haan (CBTC 1256), *H. maydis* Nisikado and Miyakel (CBTC 2314), *Penicillium digitatum* Sacc. (CBTC 1121), *P. expansum* Link (MTCC 4485), *P. italicum* Wehmer (CBTC 1029), *P. implicatum* Biourge (CBTC 1034), *P. minio-luteum* Dierckx (CBTC 1045), *P. variabile* Sopp (CBTC 1046), *Phoma violacea* (Bertd) Eveleigh (CBTC 2051), *Rhizopus nigricans* Ehrenb (CBTC 2167) (Samson, et al., 1995) were collected from Microbial type culture collection (MTCC), Chandigarh (India) and Collection of Bio-resource Type Culture (CBTC), Microbiology Department, CCS University, Meerut (India). All cultures were maintained on potato dextrose agar medium (200g scrubbed and diced potato in 1000ml distilled water, 15g agar, 20g dextrose $P^H \pm 5.6$). A seven day old culture of each fungus was used for bioactivity tests.

Isolation of active constituents: The essential oil was extracted from the fresh rhizomes of *Zingiber officinale* Rosc (Family-*Zingiberaceae*)by hydro-distillation using Clevenger's apparatus (Clevenger, 1928). A clear light yellow green coloured oily layer was separated and dried with anhydrous sodium sulphate. The physiochemical properties of the oil were determined by the technique described by Langenau (1948).

In vitro studies: The minimum bioactive concentrations (MBCs) of the oil were determined following the poisoned food technique (PFT) of Grover and Moore (1962) with slight modification (Shahi, et al, 1999). The requisite quantity of the oil was dissolved in 2-ml acetone and then added in 100ml pre-sterilized potato dextrose agar (PDA) medium (P^H-5.6). In control sets, sterilized water (in place of the oil) and 2ml acetone were used in the medium. Mycelial discs of 5mm diameter, cut out from the periphery of 7day old cultures of the test pathogens, were aseptically inoculated upside down on the agar surface of the medium. Inoculated petri plates were incubated at 27±1°C and the observations were recorded on seventh day.

Percentage of mycelial growth inhibition (MGI) was calculated as follows:

$$\mathbf{MGI\ (\%) = (dc\text{-}dt) \times 100 / dc}$$

- dc = mycelial growth diameter in control sets, dt = mycelial growth diameter in treatment sets

The nature of antifungal activity, fungistatic (temporary inhibition) / fungicidal (permanent inhibition) of the oil was determined by the method of Garber and Houston (1959). The inhibited fungal discs (at minimum bioactive concentrations) were reinoculated up side down on plain PDA (potato dextrose agar) medium in petri plate. Observations were recorded on 7th day of incubation at 27±1^{o}C. Fungal growth on 7th day indicated fungistatic action of the oil, while absence of growth indicated fungicidal action of the oil.

The effect of inoculum potentiality on minimum bioactive concentrations (MBCs) of the oil was determined by the method of Shahi et al., (1999). Mycelial disc of 5mm in diameter of seven day old cultures were inoculated in culture tube containing respective MBCs of oil in liquid medium (Potato dextrose broth) separately. In controls, sterile water were used in place of oil and run simultaneously. Observations were recorded after 7th day of incubation. Absence of mycelial growth in treatment sets on seventh day exhibited the oil's potential against heavy doses of inoculum.

Effect of physical factor: Effect of temperature and duration of toxicity during storage of the oil was evaluated according to Shahi et al., (1999). Five lots of oil were kept in small vials, each containing 5ml of oil; these were exposed at 40, 60, 80 and 100°C in an incubator for 60 minutes. Residual activity was assayed by poisoned food technique of Grover and Moore (1962). Loss of toxicity of the oil was also determined by storing the oil at room temperature (30±4°C) and withdrawn samples at intervals of 60 days up to 48months and tested by poisoned food technique (Grover and Moore, 1962). All the experiments were repeated twice and each contained five replicates; the data presented mean values.

Phytotoxic investigation: Phytotoxic effect (maximum tolerable concentration) of the oil was carried out at different concentrations (ranging from 10 to 100µl/ml) on fruits skin (epicarp) of *Malus pumilo*. Two sets of 50 samples (apples) were maintained one for the treatments and another for the controls. Each sample was first washed with distilled water followed by 70% ethyl alcohol and then allowed to dry. In treatment sets 1ml of the different concentrations of oil was sprayed to each sample separately. In controls sterilized water was sprayed (in place of oil). The qualitative observations (any observable effect on the skin (epicarp) of the fruits) have been recorded at the interval of 24h up to 3 weeks.

In vivo investigation of the oil in the form of fungicidal spray: The study was designed to see the activity of the oil in the form of fungicidal spray applied on fruit skin for the control of fruit rot of *Malus pumilo* by different methods (Shahi, et al., 2003). For *in vivo* study, both pre and post inoculation treatments (fungicidal spray) were applied to the fruits.

In the pre inoculation treatment, two set were prepared treatments as well as controls. In treatment set fruits were sprayed in known tolerable concentrations (10-50µl/ml) of oil preparation in vehicle. In controls, the fruits were sprayed with distilled water in vehicle. Thereafter, the fruits were injured using a sterilized needle, and the fungal inoculum of *Penicillium expansum, Botrytis cinerea, Phoma violacea* (5mm diameter mycelial disc of each fungus) was placed over the injured areas. All inoculated fruits were incubated at 26±1°C and the observations were recorded on seventh day.

In post inoculation treatment, fruits were first wounded with a sterilized needle and fungal inoculum of *Penicillium expansum, Botrytis cinerea, Phoma violacea* (5mm diameter mycelial disc of each fungus) was placed over the wounded areas. After 24h of incubation, fruits were sprayed in different concentrations (10-50µl/ml) of oil preparation separately. In controls, fruits were sprayed with distilled water with vehicle. Inoculated fruits were incubated at 26±1°C and the observations were recorded on seventh day. The data were average of 5 replicates and repeated twice. Percentages of inhibition (I) were calculated as follows:

$$\mathbf{I\ (\%) = (Ic - It) \times 100 / Ic}$$

- Ic= average diameter of infected area in control set, It= average diameter of infected area in treatment sets.

Statistical analysis: Two way analysis of variance (ANOVA) was used to determine the significance ($P \geq 0.05$) of the data obtained in all experiments. All results were determined to be within the 95% confidence level for reproducibility. The ANOVA was computed using the SPSS version 16.0 software package.

RESULTS

The rhizomes of *Zingiber officinale* Rosc. on hydro-distillation yielded 2.7 % essential oil. The physicochemical properties of the oil were shown in table-1. The oil exhibited broad antifungal activity, the minimum bioactive concentrations with fungistatic action (temporary inhibition) of the oil was found to be 0.4µl/ml for *Alternaria alternata*, 0.6µl/ml for *A. niger*, *A. parasiticus*, *Botrytis cinerea*, *Cladosporium cladosporioides*, *Colletotrichum capsici*, *C. falcatum*, *Fusarium cerealis*, *F. culmorum*, *Gloeosporium fructigenum*, *Penicillium expansum*, *P. digitatum*, *Penicillum italicum*, *P. implicatum*, *P. minio-luteum*, 0.8µl/ml for *Aspergillus flavus*, *A. fumigatus*,, *Curvularia lunata*, *F. oxysporum*, *F. udum*, *Helminthosporium oryzae*, *H. maydis*, *P. variable* and 1.0µl/ml for *Rhizopus nigricans* (Table-2). The bioactivity of the oil persist up to 100°C, and its did not expire even up to 48-months of storage. The oil exhibited heavy doses of inoculums (30 mycelial discs, each of 5mm diameters) at respective MBCs of the oil.

The fungicidal spray, when tested in vivo on *Malus pumilo* for checking the rotting, it showed complete inhibition at 20µl/ml concentration by pre inoculation treatment while in post inoculation treatment 30µl/ml concentration of spray solution was required for the 100% control of rotting (Table-3). The fungicidal spray was found cost effective and free from any side effects.

DISCUSSIONS

In the present investigation the oil of ginger rhizome were found effective against dominant plant pathogens.

A fungicide must not be affected by extremes of temperature. Only a few workers have studied the effect of temperature on antifungal activity of the oils, but the oil of *Pepromia pellucida* was reported to be active up to 80°C (Singh, et al., 1984); in the present study the oil of *Zingiber officinale* Rosc retained activity up to 100°C.

A substance may be fungicidal against certain fungi yet ineffective against other pathogens. Therefore, a clear picture of the toxicity of a fungicide comes only after it is tested against a large number of fungi. The literature shows that the essential oils have been found to exhibit a narrow or wide range of activity (Dubey, et al., 1983), but in the present study the *Zingiber officinale* Rosc oil exhibited a broad

antifungal spectrum. Antifungal active oils derived from plants are generally non-phytotoxic (Tripathi, et al., 1983). In the present study, the oil was found to be non-phytotoxic at morphological level. Additionally, in preliminary *in vivo* trials, it has also been found effective in the control of fruit rot of *Malus pumilo.*

A chemical should be tested under both *in vitro* and *in vivo* conditions in order to prove its potential as promising antifungals for the control of disease. Since, detailed *in vitro* studies on the essential oil of *Zingiber officinale* Rosc indicate its potential as ideal antifungal compounds against post harvest spoilage fungi; it was further subjected to *in vivo* investigation so as to confirm their efficacy as a natural product for the control of rotting in fruits. The present study clearly demonstrates that oil of *Zingiber officinale* Rosc holds a good promise as an antifungal against post harvest spoilage an account of their following virtues viz., strong efficacy against fungi with fungicidal action, potentiality against heavy fungal inoculum, long shelf life, thermostable, wide range of antifungal activity and absence of any phytotoxic effects and better result during in vivo trials. The oil in the form of fungicidal spray can be exploited commercially after undergoing successful completion of wide range of field trial and to find out their economic viability.

Acknowledgements: Thanks are due to Head, Department of Microbiology, Chaudhary Charan Singh University, Meerut for providing the facilities and for Department of Science and Technology for financial assistance.

REFERENCES

Clevenger, J.F., (1928): Apparatus for the determination of volatile oil. *Journal of American Pharmacological Association,* 17: 346.

Dubey, N.K., Kishore, N., Singh, S.N., (1983): Antifungal properties of the volatile fraction of *Melaleuca leucodendron*. *Tropical Agriculture (Trinidad),* 60: 227-228.

Garber, R.H., Houston, B.R., (1959): An inhibitor of *Verticillium albo-atrum* in cotton seed. *Phytopathology,* 49: 449-450.

Grover, R.K., Moore, J.D., (1962): Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. *Phytopathology,* 52: 876-880.

Shahi, S.K., Patra, M., Shukla, A.C., Dikshit, A., (2003): Use of essential oil as biopesticide against post harvest spoilage in fruits, *Malus pumilo*. *Bio-Control.,* 48 (2): 223-232.

Shahi, S.K., Shukla, A.C., Bajaj, A.K., Midgely, G., Dikshit, A., (1999): Broad spectrum antimycotic drug for the control of fungal infection in human beings. *Cur. Sci.,* 76: 836-839.

Simmonds, J.H., (1963): Studies in the latent phase of *Colletotrichum* species causing ripe rots of tropical fruits. *Queensland J. Agr. Sci*., 20: 373-424.

Singh, A.K., Dikshit, A., Dixit, S.N., (1984): Antifungal studies of *Peperomia pellucida*. *Beitr. Biol. Pflanzen.,* 58: 357-368.

Somasundaram, L., Coats, J.R., Racke, D.K., Stahr, H.M., (1990): Application of the Microtox system to assess the toxicity of pesticides and their hydrolysis metabolites. *Bull. Environ. Contam. Toxicol.,* 44: 254-259.

Tripathi, N.N., Dubey, N.K., Dikshit, A., Tripathi, R.D., Dixit, S.N., (1983): Fungitoxic properties of *Alpinia galanga*. *Trop. Plant Sci. Res*., 1: 49-52.

Table-1: Physico-chemical properties of the *ginger* oil.

Properties studied	Observations
Plant height (cm)	70-100
Oil yield (%)	2.7
Colour	Light yellow
Specific gravity at 25°C	0.868-0.880
Refractive index at 20°C	1.4840-1.4894
Optical rotation	-28^0-45^0
Saponification value	20 max
Solubility in 70% alcohol	1.8 vol
β-zingiberene content (%)	38.83

Table-2: Minimum bioactive concentrations of *ginger* oil against fungal pathogens.

Fungi	% mycelial growth inhibition at different concentration (µl/ml)							
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6
Alternaria alternata	54.2	100^s	100^s	100^s	100^c	100^c	100^c	100^c
Aspergillus flavus	41.2	70.6	91.2	100^s	100^s	100^c	100^c	100^c
Aspergillus fumigatus	55.5	74.3	90.1	100^s	100^s	100^c	100^c	100^c
Aspergillus niger	45.2	89.0	100^s	100^s	100^s	100^c	100^c	100^c
Aspergillus parasiticus	42.0	91.0	100^s	100^s	100^s	100^c	100^c	100^c
Botrytis cinerea	54.0	98.0	100^s	100^s	100^c	100^c	100^c	100^c
Cladosporium cladosporioides	71.0	82.2	100^s	100^s	100^c	100^c	100^c	100^c
Colletotrichum capsici	76.2	91.2	100^s	100^s	100^c	100^c	100^c	100^c
Colletotrichum falcatum	56.2	69.2	100^s	100^s	100^c	100^c	100^c	100^c
Curvularia lunata	76.2	81.0	90.2	100^s	100^s	100^c	100^c	100^c
Fusarium cerealis	67.1	90.2	100^s	100^s	100^c	100^c	100^c	100^c
Fusarium culmorum	69.2	81.2	100^s	100^s	100^c	100^c	100^c	100^c
Fusarium oxysporium	70.1	89.3	100^s	100^c	100^c	100^c	100^c	100^c
Fusarium udum	67.2	76.0	81.2	100^s	100^s	100^c	100^c	100^c
Gloeosporium fructigenum	45.2	76.2	100^s	100^s	100^c	100^c	100^c	100^c
Helmenthosporium maydis	79.2	89.2	98.9	100^s	100^s	100^c	100^c	100^c
Helmenthosporium oryzae	75.1	92.2	95.4	100^s	100^s	100^c	100^c	100^c
Penicillium digitatum	61.2	75.1	100^s	100^s	100^c	100^c	100^c	100^c
Penicillium expansum	53.1	81.2	100^s	100^s	100^c	100^c	100^c	100^c
Penicillium italicum	65.2	93.1	100^s	100^s	100^c	100^c	100^c	100^c
Penicillium implicatum	71.2	80.1	100^s	100^s	100^c	100^c	100^c	100^c
Penicillum minio-luteum	69.0	78.2	100^s	100^s	100^c	100^c	100^c	100^c
Penicillum variable	50.1	71.2	81.2	100^s	100^s	100^c	100^c	100^c
Phoma violacea	40.2	70.1	83.1	100^s	100^s	100^c	100^c	100^c
Rhizopus nigricans	60.2	81.2	91.0	92.8	100^s	100^s	100^c	100^c

- s, fungistatic action; c, fungicidal action

Table-3: *In vivo* efficacy of the *ginger* oil for the control of rotting in *Malus pumilo*.

Concentrations (µl/ml)	% inoculum growth inhibition at different treatments	
	pre-inoculation treatment	post-inoculation treatment
10	76.2	59.2
20	100	68.2
30	100	100
40	100	100
50	100	100
Variance	113.288	405.432
Std. Dev	10.6437	20.1353
Std. Err	4.76	9.0048

- This test will be performed only if K>2 and the analysis of variance yields a significant F-ratio

