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Integrated Management of Soybean (*Glycine* × *max* L. Merr.) by Essential Oil of *Citrus* × *sinensis* L cv.' Osbeck' Epicarp in Postharvest

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Abstract

The essential oil extracted from the epicarp of *Citrus sinensis* exhibited absolute fungitoxicity against soybean's fungus as *Alternaria alternate*, *Aspergillus niger*, *Cercospora kikuchii*, *Chaetomium* spp., *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium roseum*, *Macrophomina phaseolina, Penicillium italicum, Phomopsis* spp. *Rhizoctonia solani*, and *Sclerotium rolfsii*. It is very difficult to manage these pathogens by their nature of survival in seed. GC-MS studies of the oil revealed the presence of 10 chemical constituents of them limonene was found to be the major component (84.2 %). The activity of the oil was tested by the poisoned food technique and volatile activity assay. It was found that volatile activity was more toxic than poisoned food technique. The oil was extremely toxic for spore germination and it was found that at 700 ppm spore germination was inhibited in the ten test fungi out of the twelve tested. The essential oil demonstrated wide spectrum fungitoxicty. The seed were treated with essential oil for different concentration. It is evident that the treatment of essential oil inhibited the growth of dominant fungi. In 600 ppm *Fusarium roseum*, *Penicilluim italicum, Rhizocotonia solani* and *Sclerotium rolfsii* were inhibited but in 700 ppm all tested fungi who is found in untreated seed were inhibited. The scanning electron microscopy (SEM) was done to study the mode of action of oil in case of *Aspergillus niger* and it was observed that the treatment of oil leads to the distortion and thinning of hyphal wall and the reduction in hyphal diameter and absence of conidiophores.

Keywords: *Citrus sinensis*, Fungitoxicity, GC-MS, *Glycine* × *max* L. Merr, Seed treatment, SEM.

INTRODUCTION

Nowadays, throughout the world search is going on the environmentally safe, non toxic and economically viable plant based products for remedy of various plant diseases. The majority of the synthetic antimicrobial products used in agricultural purpose are toxic to different biological system as well as environment and they induce the development of resistant strains affecting plant health. Soil borne plant pathogens are sometimes difficult to control with chemical fungicides or bactericides and the application of biocides may be one of the best alternatives in controlling pathogens.

Plants are a rich source of bioactive organic chemicals. Although only some 15000 secondary plant metabolites have been chemically identified, their total number may exceed 4,000,000. They are a vast cornucopia of defense chemicals, comprising repellents feeding deterrents growth inhibition sterilants, toxicants and anti microbial agents (Sharma, 2001)

Phyto-compounds are expected to be for more advantageous than chemicals for sheer magnitude of complexity, diversity, novelty of chemicals and their reactions (Sharma, 1998). As they are biodegradable in nature, non-pollutant and posses no residual or phytotoxic properties these natural products have the potential to be safe fungicides to replace the synthetic ones. The general antifungal activity of essential oils is well documented (Meepagala *et al.*, 2002). Some of the essential oils have been reported to inhibit postharvest fungi in *in vitro* conditions (de Billerbeck *et al.*, 2001; Hidalgo *et al*., 2002; Sharma and Verma, 2004).The potential of essential oils to control postharvest decay has also been examined by spraying and dipping the fruit and vegetables (Dixit *et al*., 1995).

However, there are very few reports on antifungal activity of the *Citrus sinensis* essential oil against different microbial species (Shukla *et al*., 2000). Effects of citrus oils on the growth and aflatoxin production by *Aspergillus parasiticus* was reported by Karapinar (1985).Similarly the effect of essential oil on the growth and morphogenesis of *Aspergillus niger* was also seen (Sharma and Tripathi, 2006a). Ernestina *et al*., (2003) also reported fungicidal activity of citrus oil against the causal agent of anthracnose disease on the tropical fruits. An added advantage of some of the essential oils is their bioactivity in the vapour phase, a characteristic which makes them attractive as possible fumigants for stored product protection (Bishop and Thornton, 1997). These findings thus indicate the possibility of exploiting essential oil extracted from epicarp of *Citrus sinensis* as an effective inhibitor of the postharvest pathogens of perishables (Sharma and Tripathi, 2006b).

Soybean (*Glycine* × *max* L. Merr.) has received a great deal of attention all over the world as an important source of protein to alleviate the protein deficiency. It is comparatively cheaper than the animal sources of protein such as meat, fish, milk, egg etc. It contains 40-45% protein, 18-20% edible oil, 24-46% carbohydrate and a good amount of vitamins (Kaul and Das, 1986). As legume crop it is capable of utilizing atmospheric nitrogen through biological nitrogen fixation. Soybean fixes about 270 kg N/ha compared to 58 to 157 Kg N/ha by other pulses (Haque, 1978). As a result the crop is less dependent on chemical nitrogenous fertilizers. Most of the people of India now consume soybean oil in their daily dishes due to its high quality. A large number of soya products are successfully developed and some of them such as soyaflour, soyamilk, soyabicuits, soyabread, soyachanachur etc are commercially produced in India. At present the total acreage under this crop is about 5000 hectares and total production is about 3750 tons all over the country (BBS, 1998).

All parts of the soybean plant are susceptible to diseases. More than 100 pathogens are known to affect soybean. (Sinclair and Backman, 1989). Soybean diseases reduce yield, on an average of 10 to 30% in most production area (Sinclair 1994). No exact data on yield losses due to diseases of soybean is available in India. The total yield loss due to disease during 1994 in 10 countries with greatest soybean production was 28.5×10^6 metric tan, valued at U.S. $\$6.29 \times 10^9$

million (Wrather *et al*., 2001). More than 40 species of phytopathogenic fungi, bacteria and viruses may infest soybean seed causing various diseases, out of which 15 can result in significant economical losses, reducing yield and deteriorating quality of seed crop. Infected seed can provide primary inoculum for infestation of new crop and seed-borne pathogens may be dispersed for long distances with it (Hartman *et al*., 1999)

Methods for complete control of soybean seed borne diseases are yet to be developed. Management strategies for these diseases include use of presumed disease free seeds, resistant cultivars and fungicidal sprays. Seed treatment is one of the best methods to manage seed-borne diseases. The continuous and indiscriminate use of chemicals to manage the crop disease results in accumulation of harmful chemical residues in the soil, water and grains. Development of fungicide-resistant biotypes of the pathogens is a major constraint to control the major seed-borne pathogens of soybean. In recent years, considerable success has been achieved by introducing antagonists to control seed-borne fungal pathogens. A considerable work has been done in controlling seedling diseases of many crops caused by *Rhizoctonia solani* and *Sclerotium rolfsii* both *in vitro* and pot culture experiments by using Trichoderma (Pradeep *et al*., 2000; Haider, 2005). Some plant extracts also found to be most effective in reducing the growth and development of many pathogens (Raihan *et al*., 2003; Begum and Bhuiyan, 2006).

The present study has therefore been undertaken with the objective to evaluate the efficacy of essential oil against the major seed-borne pathogens of soybean.

MATERIALS AND METHODS

Seed Source

Seed samples were obtained from the Daliganj market. Lucknow. One variety of soybean was used as VL Soya 21.

Isolation Techniques

Two generalized isolation procedures were employed for the isolation of pathogenic and saprophytic fungi (Neergaard, 1977). The two methods were the moist blotter and the Potato dextrose agar (PDA) method.

Isolation on Moist Blotting Paper

Ten non-sterilized seeds were evenly placed on three layers of moistened 9 cm diameter filter paper (Whatman No.1) in plastic Petri dishes to allow for the penetration of light. A total of 10 seeds were used for each sample. Total 50 seeds are taken. The plates were incubated at 27±2°C for 4 to 5 days in an alternating cycle of 12 hours NUV (near ultra violet light) and 12 hours darkness regime. Fungi developing on seeds were examined and transferred to PDA for identification and pathogencity studies.

Incubation on PDA

Ten seeds surface sterilized for 10 minutes in 1 percent solution of sodium hypochlorite as a pre - treatment were evenly spaced on PDA medium. The plates were incubated at 27 ± 2°C for 4 to 5 days in an alternating cycle of 12 hours NUV and 12 hours darkness. A total of 10 seeds per sample were used. Fungi developing on seeds were identified as in the previous experiment.

Essential Oil

The fresh epicarp of *Citrus× sinensis* L. cv.‘ Osbeck’ (Rutaceae) was collected from various juice shops of Lucknow District, Uttar Pradesh, India during the months of May to October 2008. The essential oil was extracted from the collected materials by hydro-distillation for 5 hrs using the Clevenger-type apparatus (Guenther, 1948). A clear, light yellow coloured, oily layer was ob-

tained on top of the aqueous distillate which was separated from the latter and dried with the anhydrous sodium sulphate. The extracted essential oil was kept in air-tight sealed glass vials and covered with aluminum foil at 4oC until further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oil

The GC-MS of the essential oil was analyzed on a Shimadzu QP-2000 instrument at 70 eV and 250°C. GC Column: ULBON HR-1 equivalent to OV-1, fused silica capillary – 0.25 mm×50 M with film thickness - 0.25 µ. The GC-Mass was operated under the following conditions 60-5-5-250 meaning that the initial temperature was 60°C for 5 minutes and then heated at the rate of 5°C per minute to 250°C. The carrier gas (Helium) flow was 2 ml per minute.

The identification of component was based on the comparison of their mass spectra fragmentation patterns with those of Mass Spectrometry Data Centre, the Royal Society of Chemistry. U.K. (Eight Peak Index of Mass Spectra, 3rd Ed. 1983) and those reported in the relevant literature (Adams, 1995).

Fungitoxicity Assay

The antifungal activity was tested against the test pathogens by poisoned food technique and volatile activity assay. The fungi-toxicity of the oil was evaluated against the test fungi by the poisoned food technique of Grover and Moore (1962). PDA (20 ml) was poured into sterilized petri dishes (90 mm diameter) and measured amount of oil was added to give desired concentrations (25, 50, 100, 200, 300, 400, 500, 600 and 700 ppm). In media 0.05 % Tween-80 was added for even distribution of the oil in the medium. The medium was supplemented with the same amount of distilled water instead of oil for the control sets. The test fungi were incubated at 27±2°C. On the 7th day, the growth of the test fungi was recorded and the percentage inhibition was computed after comparison with the control.

Tests for the volatile activity of oil were carried out in the 90 mm petri plates containing 20 ml of solidified PDA. A 5 mm diameter disc of inoculum of the test species, cut from the periphery of an actively growing culture on PDA plates, was placed on the agar in each petri plate and was kept in the inverted position. In the upper lid of petri plates sterilized cotton swab was placed and in that different concentration of oil were poured and the plates were sealed by parafilm to check the release of the volatile oil. For each corresponding control equal amount of water was poured on the sterilized cotton swab. The petri plates were kept at 27±2°C for 7 days.

Fungitoxicity was expressed in terms of percentage of mycelia growth inhibition and calculated as per formula of Pandey *et al*., (1982):

$$\text{Percentage of mycelial growth inhibition} = \frac{dc-dt}{dc} \times 100$$

Where dc = Average diameter of fungal colony in control
dt = Average diameter of fungal colony in treatment

Spore Germination Assay

In the spore germination assay, ten concentrations of oil (25, 50, 100, 200, 300, 400, 500, 600 and 700 ppm) were tested against each test fungi viz *Alternaria alternate*(Aa), *Aspergillus niger*(An), *Cercospora kikuchii*(Ck), *Chaetomium* spp(Cspp)., *Cladosporium cladosporioides*(Cc), *Curvularia lunata*(Cl), *Fusarium roseum*(Fr), *Macrophomina phaseolina*(Mp), *Penicillium italicum*(Pi), *Phomopsis spp*(Pspp.), *Rhizoctonia solani*(Rs), and *Sclerotium rolfsii*(Sr). The fungal spores obtained from 10-day-old cultures of the fungi were taken and placed on the glass slides in triplicate. The slides containing the spores were incubated in a moist chamber at 27 ± 2° C for 24

hrs. Each slide was then fixed in lactophenol-cotton blue and observed under the microscope for germination of the spores. About 200 spores were counted and the number of spores germinated was scored to calculate the percentage of the spore germination (Surender *et al*., 1987).

Seed Treatment with Essential Oil

Seed treatment of soybean was assumed as volatile assay. So the volatile activity of oil was carried out in the 90mm petri plates containing 20 ml of solidified PDA. 4 seeds were placed on the agar in each petri plate and were kept in the inverted position. In the upper lid of petri plates sterilized cotton swab was placed and in that different concentration of essential oil (25, 50, 100, 200, 300, 400, 500, 600 and 700 ppm) were poured and the plates were sealed by parafilm to check the release of the volatile oil. Treated seed with essential oil for above concentrations were incubated in potato dextrose agar (PDA) at 27 ± 2°C for 4 to 5 days .They were studied for the growth of fungal from the seed surface.

RESULTS

The most common fungi found to be growing on all untreated seed were *Alternaria alternate*(Aa), *Aspergillus niger*(An), *Cercospora kikuchii*(Ck), *Chaetomium* spp(Cspp)., *Cladosporium cladospori*oides(Cc), *Curvularia lunata*(Cl), *Fusarium roseum*(Fr), *Macrophomina phaseolina*(Mp), *Penicillium italicum*(Pi), *Phomopsis* spp(Pspp.), *Rhizoctonia solani*(Rs), and *Sclerotium rolfsii*(Sr).

The epicarp of *C. sinensis* on hydro-distillation yields 1.8 % essential oil which was pale yellow in colour with strong and fragrant odour. A typical GC-MS chromatogram of the essential oil is shown in Fig. 1 while the results of the quantitative analysis are presented in Table 1. The GC-MS studies of the oil revealed the presence of 10 chemical constituents. The most abundant component in the investigated oil was Limonene (peak 4, 84.2 %) followed by linalool (4.4%) and myrcene (4.1%) (Fig. 1, Table 1).

The fungitoxicity of the oil was measured by percent radial growth inhibition using poisoned food technique and volatile activity assay. As it is evident from Table 2, out of twelve postharvest pathogens tested, except *Phomopsis* spp rest were inhibited by *C. sinensis* oil. In poisoned food technique the MIC was found to be 500 ppm for *Alternaria alternate*, *Cercospora kikuchii*, *Chaetomium* spp., *Cladosporium cladosporioides* and *Penicillium italicum* .All tested fungi showed maximum inhibition in 600 ppm except *Fusarium roseum*, *Phomopsis* spp., *Rhizoctonia solani*, and *Sclerotium rolfsii*. All most fungi inhibited in 700 ppm except *Phomopsis* spp., It was observed that in volatile assay MIC was beginning to 300 ppm in *Cercospora kikuchii*. A most twelve pathogen inhibited essential oil of concentration of 400 ppm to 700 ppm except *Phomopsis* spp., however, 700 ppm was the dose to sufficient to check the growth in essential oil activity assay.

In the spore germination study only ten pathogens tested against essential oil. At 400 ppm concentration of oil there was complete inhibition of spore germination in *Cercospora kikuchii*. In case of *Aspergillus niger*, Cladosporium cladosporioides and Curvularia lunata spore germination was inhibited at 500 ppm, 600 ppm concentration checked spore germination in case of all fungi except Fusarium roseum, Penicillium italicum. However 700 ppm was required to check the spore germination of all tested fungi. It was also observed that those spores which germinated in presence of low concentrations of oil produced small germ tubes as compared to the control (Table 3) .

Investigations were also carried out to study the mode of action of the essential oil from Citrus ×sinensis epicarp against A. niger. As this fungus is opportunistic by nature, it is capable of growing on wide range of organic substrates causing biodeterioration of stored products. The effects of *C. sinensis* essential oil on the morphological changes in A. niger were examined by SEM and are shown in Fig. 2a and 2b. In control, the A. niger mycelium grown on PDA medium pre-

sented regular, homogenous hyphae with smooth cell walls and clear development of conidiophore (Figure. 2a). However, presence of essential oil in the culture medium (400 ppm) showed clear absence of conidiophore and induced cell wall modifications (Figure. 2b).

The observation with seed treated with the essential oil reveal that low amount of treatment was effective. Only *Cercospora kikuchii* was inhibited in 400 ppm. In 500 ppm Alternaria alternate and Cladosporium cladosporioides are inhibited but all not. In 600 ppm Fusarium roseum , Penicilluim italicum, Rhizocotonia solani and Sclerotium rolfsii were inhibited but in 700 ppm all tested fungi who is found in untreated seed were inhibited .The result indicated that the not high amount of essential oil of seed treatments was effective in controlling the growth of the entire surface borne seed mycoflora (Table 4).

DISCUSSION

More than 40 species of phytopathogenic fungi, bacteria and viruses may infest soybean seed causing various diseases, out of which 15 can result in significant economical losses, reducing yield and deteriorating quality of seed crop. Infected seed can provide primary inoculum for infestation of new crop and seed-borne pathogens may be dispersed for long distances with it (Hartman *et al*., 1999). Soybean seed-borne diseases occur in the Vojvodina province, too (Vidić *et al*., 2003, Ignjatov *et al*., 2006, Medić-Pap, 2007). Recently soybean becomes one of the most important crops in the province. That is why this work, aiming at the assessment of seed infection level in the most often grown cultivars was carried out.

Citrus sinensis essential oil exhibited the broad spectrum of fungicidal/fungistatic activity against the test organisms. The MIC against test pathogens in volatile activity assay (400 to 500 ppm) was lower in comparison to that observed in poisoned food technique (500 to 700 ppm). Essential oils previously tested against various pathogens have been reported to exhibit higher MIC values as compared to MIC values stated in this study (Pandey *et al*., 1982; Beg and Ahmad, 2002; Nguefack *et al*., 2004). The difference in the values for MIC obtained with the essential oil show that the level of antimicrobial activity of essential oil is closely dependent on the screening methods used (Delespaul *et al*., 2000). The general antifungal activity of essential oils and their effects on postharvest pathogens in *in vitro* conditions are well documented (Bishop and Reagan 1998; Hidalgo *et al*., 2002).

The effect of *C. sinensis* oil on inhibition of spore germination indicates that 300 to 500 ppm concentration is sufficient to check spore germination in most of the pathogens tested. This concentration is less than many other essential oils previously tested by various workers (Beg and Ahmad, 2002). Similar results have been reported by using different testing methods (Pattnaik *et al*., 1996). Soliman and Badeaa (2002) reported complete inhibition of *Aspergillus flavus*, A. parasiticus and A. ochraceus by the oils of thyme and cinnamon (<500 ppm), marigold (<2000 ppm), spearmint, basil (3000 ppm). Treatment with 500 ppm concentration exhibited 70 % to 100 % inhibition of the spore germination in most of the fungi tested.

The effects of *C.* × *sinensis* oil on the morphology of *Aspergillus niger* hyphae examined by SEM revealed alterations in the morphology of the hyphae, which appeared severely collapsed and squashed due to lack of cytoplasm. Our observations find support from the findings of the surface modifications in SEM study as observed by de Billerbeck *et al*. (2001) using *Cymbopogon nardus* essential oil against *A. niger*. Zambonelli *et al*., (1996) reported similar findings in *Pythium ultimum* and *Colletotrichum lindemuthianum* treated with thyme and lavender oil. Such modifications induced by essential oils may be related to the interference of essential oil components with enzymatic reactions of wall synthesis, which affects fungal morphogenesis and growth. Zambonelli *et al*., (1996) reported fungal growth inhibition associated with degeneration of fungal hyphae after treatment with *Thymus vulgaris* essential oil. The present piece of work is an attempt to standardize *Citrus*× *sinensis* essential oil based on its fungitoxic property against postharvest

pathogens. The citrus oil as fungitoxic agent present two main characters, the first is its natural origin which means more safety to people and environment and the second is that it has to be considered at low risk for resistance development by postharvest pathogens. It is believed that it is difficult for the pathogens to develop resistance to such a mixture of oil components with apparently, different mechanism of antifungal activity.

Application of essential oil for the control of seed borne disease is method devoid of any health hazard problem. The antifungal effect of selected oil can be applied at a larger scale to treat the seed before sawing them in the field. The oil being of plats origins has hazardous effects on the seeds as well as on soil. The seed treatment with oil does not have any adverse effect on the germination of seed even after the treatments. So the seed treatment of oil will not create any problem of pollution and the chemicals of plants oil easily degrade in the soil.

Essential oil of *Citrus sinensis* showed a wide spectrum of fungicidal activity, which indicating that the active component could possibly be an ester. Results signify the potentiality of *Citrus sinensis* as a source of antifungal therapies and hence, further work is required to evaluate its potentially active component on other pathogens as this biofungicidal botanics is environmentally safe and could replace the toxic and hazardous synthetic compounds. Simultaneously investigations are also needed to characterize and formulate the active component of the extract which may provide lead for the discovery of a novel antifungal compound from *Citrus sinensis.*

Literature Cited

Adams, R.P. 1995. Identification of essential oil components by gas chromatography mass spectroscopy; Allured Publishing Corporation: Carol Stream, Illinois.

Bangladesh Bureau of Statistics (BBS). 1998. Statistical yearbook of Bangladesh 1997. Ministry of Planning, Govt. of the P.R. of Bangladesh, Dhaka.

Beg, A.Z. and Ahmad, I. 2002. *In vitro* fungitoxicity of the essential oil of Syzygium aromaticum. World J. Micro and Biot. 18, 313-315.

Begum, F. and. Bhuiyan, M.K.A. 2006. Integrated control of seedling mortality of lentil caused by Sclerotium rolfsii. Bangladesh J. Plant Path. 23: 60-65.

Bishop, C.D. and Reagan, J. 1998. Control of storage pathogen Botrytis cinerea on Dutch white cabbage (Brassica× oleracea var. capitata) by essential oils of Monarda ×citriodora var. citriodora and Melaleuca ×alternifolia on the postharvest pathogens. J. Ess. Oil Resh. 10, 57-60.

De Billerbeck, V. G., Roques, C. G., Bessiere, J. M. Fonvieille, J. L. and Dargent, R. 2001. Effects of *Cymbopogon× nardus* L. W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. Cana. J. Micro. 47: 9-17.

Delespaul, D., de Billerbeck, V.G., Roques, C.G., Michel, G., Marquier-Vinuales, C. and Bessiere, J.M. 2000. The antifungal activity of essential oils as determined by different screening methods. J. Ess. Oil Resh. 12: 256-266.

Dixit, S.N., Chandra, H., Tiwari, R. and Dixit, V. 1995. Development of botanical fungicides against blue mold of mandarins. J. Stored Prod. Resh. 31: 165-172.

Ernestina, A., Miguel, A.M., Maria, M. H., Socorro, V., Eduardo, P. and Irasema, V. 2003. Fungicidal potential of methoxylated flavones from citrus for *in vitro* control of *Colletotrichum gloeosporioides*, causal agent of anthracnose disease in tropical fruits. Pest Mgt. Sci. 59 (11):1245-1249.

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

Guenther, E. 1948. The essential oils Vol.1 pp 774, D. Van Nostrand Co, New York, NY Princeton York Allured Publication, Carol Stream, IL.

Haider, M.M. 2005. Biological and chemical control of *Rhizoctonia* dry root rot and foliar blight of soybean (*Glycine* × *max* L. Merr). Department of Plant Pathology, Bangabandhu Sheik

Mujibur Rahman Agricultural University, Gazipur. 68 pp
Haque, M. S. 1978. Present availability of nitrogen from organic wastes and biological sources. Seminar on Nitrogen in Crop Production. December-January, 1977-1978, Dhaka.
Hartman, L., Sinclair, J.B. and Rupe, J.C. 1999. Compendium of soybean diseases. APS Press, St. Paul, Minnesota.
Hidalgo, P.J., Ubera, J.L., Santos, J.A., LaFront, F., Castelanos, C., Palomino, A. and Roman, M. 2002. Essential oils in *Culamintha*× *sylvatica*. Bromf. Ssp. Ascendens (Jorden) P.W. Ball wild and cultivated productions and antifungal activity. J. Ess. Oil Resh. 14: 68-71.
Ignjatov, M. Balaz, J. Milosevic, M. and Popovic, T. 2006. Bakteriozna pegavost soje. Arh. Poljopr. Nauke 67(239): 11–17.
Karapinar, M. 1985.The effects of citrus oils and some spices on growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999. Intl J. Food Micro. 2(4):239-245.
Kaul, A. K. and Das, M. L. 1986. Oilseeds in Bangladesh. Bangladesh Canada Agriculture Sector Team. Ministry of Agriculture, Govt. of the People's Republic of Bangladesh, Dhaka. p.324
Medic-Pap, S. 2007. Mycoflora – the cause of soybean seed diseases. Typescript. University of Novi Sad, Novi Sad.
Meepagala, K.M., Sturtz, G. and Wedge, D.E. 2002. Antifungal constituents of the essential oil fraction of *Artemisia* × *drancunculus* L. var. Dracunculus. J. Agri, and Food Chem. 50: 6989–6992.
Neergaard, P. 1977 . Seed pathlogy Vol I. and II McMillan Press Itl. Londaon :Pp 1187.
Nguefack, J. Leth, V. AmvamZollo, P.H. and Mathur, S.B. 2004. Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. Intl. J. Food Micro. 94: 329-334.
Pandey, D.K. Tripathi, N.N. Tripathi, R.D. and Dixit, S.N. 1982. Fungitoxic and phytotoxic properties of the essential oil of *H.* × *suaveolens*. Zeit. Pflazenkran. Pflanzensch 89: 344-349.
Pattnaik, S. Subramanyam, V.R. and Kole, C. 1996. Antibacterial and antifungal activities of ten essential oils *in vitro.* Microbios 86(349): 237-246.
Pradeep, K., Anuja Kumud, K., Kumar, P. and Kumar, K. 2000. Biocontrol of seed borne fungal pathogens of pigeonpea (*Cajanus* × *cajan* L. Millsp.). Ann. Plant Prot. Sci.8:30-32.
Raihan, M.G., Bhuiyan, M.K.A. and Sultana, N. 2003. Efficacy of integration of an antagonist, fungicide and *In vitro* control of *Colletotrichum dematium* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum* garlic extract to suppress seedling mortality of peanut caused by *Rhizoctonia solani* and *Sclerotium rolfsii*. Bangladesh J. Plant Path.19 (1and2): 69-73.
Sharma, N. and Verma, U.K. 2004. Bioactivity of *Hyptis*× *suaveolens* on storage mycoflora. *In:* International Conference on Fumigation and Controlled Atmosphere. pp. 55 Conrad Jupiters, Gold Coast, Australia.
Sharma, N. 1998. Control of postharvest diseases with natural plant products. In :Postharvest Diseases of Horticultural Perishables. pp. 1-27 Ed. Neeta Sharma and M.M. Alam. International Book Distributing Company, Lucknow 226 004 (India) ISBN 81-85860-21-1
Sharma, N. and Tripathi, A. 2006a. Effect of *Citrus sinensis* L. cv. 'Osbeck' epicarp essential oil on the growth and morphogenesis of *Apergillus niger* L. var. 'Tieghem'. Microbiological Research.163 (3) 1: 337-344
Sharma, N. and Tripathi, A. 2006b. Fungitoxicity of the essential oil of *Citrus sinensis* on the postharvest pathogens. World J. Micro and Biot. 22:587-593
Sharma, N. 2001. Preservation of dried fruits and nuts from biodeterioration by natural plant volatiles *In:* Proceedings of an International Conference on Controlled Atmosphere and Fumigation in Stored Products, (Edited by: Donahaye, E.J., Navarro, S. and Leesch, J.) Fresno, California. Executive Printing Services, Clovis, CA USA, pp. 195-208.
Shukla, A.C. Shahi, S.K. and Dixit, A. 2000. Epicarp of *Citrus* ×*sinensis*: a potential source of natural

pesticides. Indian Phyto.53 (4): 468-471.
Sinclair, J.B. 1994. Reducing losses from plant diseases. World Soybean Research Conference V. Abstracts 10 p.
Sinclair, J.B. and Backman, P.A. 1989. Compendium of soybean disease. The American Phytopathological Society, 3rd Edn. viii+106pp.
Soliman, K.M. and Badeaa, R.I. 2002. Effect of oil extracted from some medicional plants on different mycotoxigenic fungi. Food and Chem. Tech. 40, 1669-1675.
Surender, P., Janalah, C., Reddy, V.K. and Reddy, S.M. 1987. Antifungal activity of secretions of scent glands from *Heteropteram* bugs. Indian J. Expt. Bio. 25: 233-234.
Vidic, M., Jasnic, S. and Dorovic, V. 2003. Bolesti semena soje. Biljni Lekar 6: 585–591.
Wrather, J. A. T. R. Anderson, D. M. Arsyad, Y. Tan, L.D. Ploper, A. Porta-Puglia, H.H. Ram and J. T. Yorinori. 2001. Soybean disease loss estimates for the top ten soybean-producing countries in 1998. Can. J. Plant Pathol. 23: 115–121
Zambonelli, A., Zechini, D., Aulerio, A., Bianchi, A. and Albasini, A. 1996. Effects of essential oil on phytopathogenic fungi. Phytopathology.144: 491-494.

Tables

Table 1. Components of *Citrus sinensis* L. cv. 'Osbeck' epicarp essential oil identified by GC-MS.

Peak No.	Components	Retention time (scan)	Percentage in total oil
1	α-pinene	12.36 (192)	0.9
2	β-pinene	13.70 (232)	0.6
3	Myrcene	14.60 (259)	4.1
4	**Limonene**	16.60 (319)	**84.2**
5	Linalol	18.60 (379)	4.4
6	Citral	19.96 (420)	0.5
7	α-Terpineol	21.10 (454)	0.8
8	Terpinolene	21.56 (468)	1.3
9	Citronellal	22.93 (509)	1.9
10	Geraniol	23.86 (537)	1.3

Table 2. Effect of different concentrations of Citrus sinensis oil on percent radial growth inhibition by poisoned food technique (PF) and volatile activity (VA) assay of different test fungi at 27±2°C.

(ppm)		Percent radial growth inhibition											
		Aa	An	Ck	Cspp.	Cc	Cl	Fr	Mp	Pi	Pspp.	Rs	Sr
Control	PF	-	-	-	-	-	-	-	-	-	-	-	-
VA		-	-	-	-	-	-	-	-	-	-	-	-
25	PF	8.6	7.6	15.2	5.6	5.7	4.2	5.9	3.1	3.2	2.6	4.8	5.9
VA		12.3	11.2	21.3	9.3	15.6	13.6	12.9	10.6	11.6	8.9	9.8	12.9
50	PF	16.2	14.3	31.2	14.6	13.2	12.6	14.1	8.8	13.4	8.9	18.2	14.1
VA		20.1	23.3	39.0	20.3	31.3	21.8	26.5	14.3	20.6	15.6	20.6	26.5
100	PF	28.3	29.5	49.2	29.6	28.9	25.3	31.4	15.6	23.7	12.3	30.3	31.4
VA		35.6	37.3	52.3	35.6	43.6	36.9	41.2	26.4	43.8	21.3	35.3	41.2
200	PF	49.8	51.2	65.2	39.2	44.6	41.2	47.0	29.2	46.3	25.4	46.6	47.0
VA		59.3	62.3	72.3	46.2	54.6	48.3	59.6	39.2	62.5	39.2	56.8	59.6
300	PF	55.3	56.3	85.3	49.3	67.2	60.1	63.0	41.2	63.2	46.1	57.0	63.0
VA		64.2	68.2	100.0	62.3	83.6	71.6	80.1	53.1	79.1	51.2	69.4	80.1
400	PF	74.2	85.2	100.0	68.2	89.0	73.2	78.8	59.1	81.2	62.5	81.3	78.8
VA		92.0	100.0	100.0	79.2	100.0	100.0	96.3	68.9	100.0	69.2	86..3	96.3
500	PF	86.0	100.0	100.0	82.3	100.0	100.0	83.2	74.2	100.0	69.3	85.2	85.3
VA		100.0	100.0	100.0	100.0	100.0	100.0	97.2	100.0	100.0	78.2	89.7	100
600	PF	100.0	100.0	100.0	100.0	100.0	100.0	94.3	100.0	100.0	75.2	96.2	95.2
VA		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	86.2	100.0	100.0
700	PF	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	84.3	100.0	100.0
VA		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	94.5	100.0	100.0

Table 3. Effect of different concentrations of Citrus sinensis oil on inhibition of spore germination of ten postharvest pathogens at 27±2°C.

(ppm)	Percent radial growth inhibition									
	Aa	An	Cc	Ck	Cl	Csp	Fr	Mp	Pi	Rs
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
25	72.3	71.2	73.2	68.2	74.3	73.6	78.2	98.0	86.2	72.3
50	57.2	41.2	48.2	36.2	68.2	44.8	54.3	85.4	65.2	61.2
100	41.2	28.2	22.3	20.3	44.5	31.2	48.2	61.2	53.2	57.2
00	20.3	11.3	9.2	10.2	29.3.	19.4	35.6	48.2	38.2	35.2
300	14.2	5.3	4.4	2.5	10.2	12.3	21.3	39.2	25.2	18.2
400	9.2	1.1	1.2	0.0	3.2	4.6	9.8	15.2	21.3	7.4
500	1.2	0.0	0.0	0.0	0.0	1.2	5.2	5.2	12.2	2.3
600	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	4.2	0.0
700	0.0	0.0	0.0	0.0	0.0	0.0	0.0	00	0.0	0.0

Table 3. Effect of different concentrations of Citrus sinensis oil on inhibition of spore germination of ten postharvest pathogens at 27±2°C.

	Mycoflora on treated seed with essential oil (in ppm)								
	25	**50**	**100**	**200**	**300**	**400**	**500**	**600**	**700**
Aa	Aa	Aa	Aa	Aa	Aa	Aa	-	-	-
An	An	An	An	An	An	An	An	-	-
Cc	Cc	Cc	Cc	Cc	Cc	Cc	-	-	-
Ck	Ck	Ck	Ck	Ck	Ck	-	-	-	-
Cspp.	Cspp	Cspp	Cspp	Cspp	cspp	Cspp	Cssp.	-	-
Cl	Cl	Cl	Cl	Cl	Cl	Cl	-	-	-
Fr	Fr	Fr	Fr	Fr	Fr	Fr	Fr	Fr	-
Mp	Mp	Mp	Mp	Mp	Mp	Mp	Mp	-	-
Pi	Pi	Pi	Pi	Pi	Pi	Pi	Pi	Pi	-
Pspp.	Pspp.	Pspp.	Pspp.	Pspp.	Pspp.	pspp	pspp	-	-
Rs	Rs	Rs	Rs	Rs	Rs	Rs	Rs	Rs	-
Sr	Sr	Sr	Sr	Sr	Sr	Sr	Sr	Sr	-

Figures

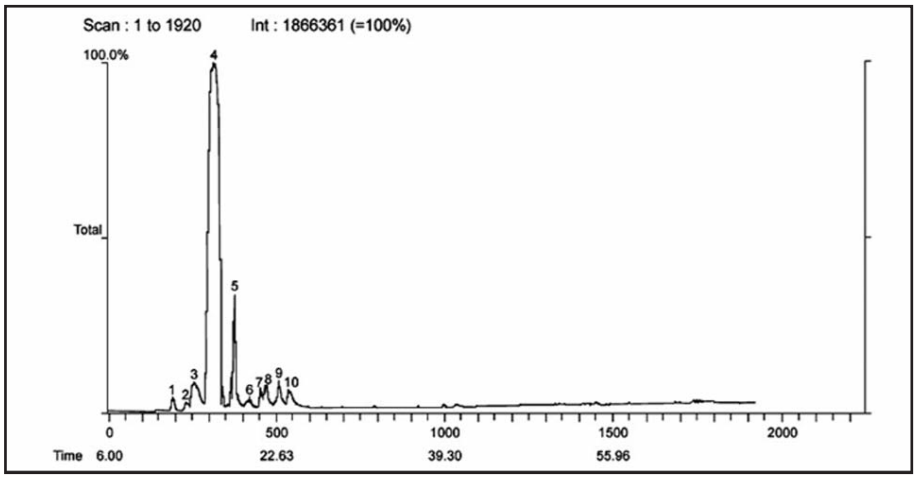

Fig. 1. GC MS Chromatogram of *Citrus sinensis*. L cv. 'Osbeck' essential oil. peak 4, showing major component, Limonene (84.2 %).

Fig. 2. (a) *Aspergillus niger* control after 5 days of inoculation at 27± 2 °C showing clear mycelium and conidial heads on conidiophores.

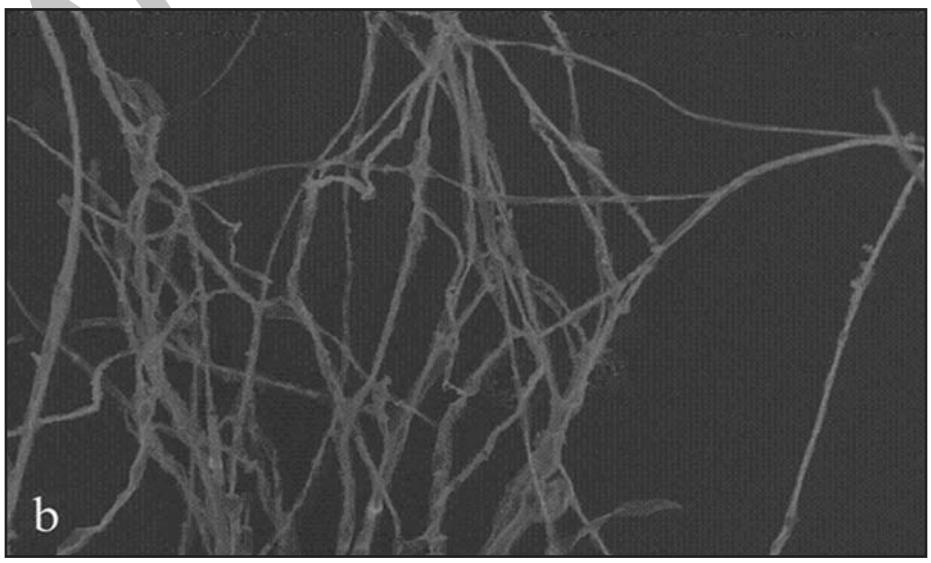

Fig. 2. (b) *Aspergillus niger* treated with 400 ppm citrus oil after 5 days of inoculation at 27±2 °C showing distorted mycelium, cell wall disruption and squashed hyphae.