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Antifungal Activity of Lemongrass (*Cymobopogon citratus*) Extracts, Biocontrol Agents to Control the Root Rotting Fungi in Brinjal Plant

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Abstract. Lemongrass (*Cymobopogon citratus*) is used as a biocontrol agent to suppress the root rotting fungi and ultimately enhance the growth of brinjal (*Solanum melongena*) plant. During *in vitro* experiment n-hexane, chloroform and methanol i.e. extracts at different concentrations 10 µL, 20 µL, 30 µL and 50 µL of lemongrass were checked against different fungi like, *Rhizoctonia solani, Fusarium solani, F. oxysporum* and *Macrophomina phaseolina.* Application of n-hexane at 50 µL showed highly significant inhibitory zone against all test fungi, while during field experiment the sharp increase in root and shoot length 5% w/v mixture of lemongrass powder and it was applied in Brinjal field. *M. phaseolina* along with other root rotting fungi like, *R. solani*, *F. solani* and *F. oxysporum*.

Keywords: brinjal, biocontrol, crop, pathogens, root rot, solvent

Introduction

Brinjal a among important vegetable crops belongs to family Solanaceae is grown throughout Asia (Prodhan *et al*., 2018). It is grown for its purple edible fruit and spongy fruit of the plant is considered as vegetable and it is widely used in cooking in many cuisines. However, the botanical definition brinjal is a berry. Brinjal is ubiquitous in distribution especially in Pakistan, Bangladesh, Sri Lanka, Nepal and India (Biswas *et al.*, 2018) consumed it in their daily meals.

Brinjal is cultivated in Pakistan in 9,000 ha areas and production is 87,000 tons annually. (FAO, 2014). Among vegetables Brinjal is of significant importance with essential nutrients and it is a rich composition of dietary fiber, potassium and sugar and has low in protein, carbohydrate and fats (Kumar and Chopra, 2016). Moreover, extract of brinjal plant helps in reduction of cholesterol levels. (Kumar and Chopra, 2016; Kumar, 2015) Minor alteration in composition of nutrients occurs with change in seasons and environment of cultivation. Besides, brinjal has also been extensively used in traditional medicine (Kudlu and Stone, 2013; Kashyap *et al*., 2003).

Brinjal cultivation is severely affected due to root diseases which caused by root rotting fungi like *Fusarium* spp., *R. solani* etc. These pathogens are very damaging, especially in hot and humid conditions (Altinok and Can, 2010). Moreover, market value of such crops reduces because of shortens shelf life. Present methods for control of this infection involve application of various chemicals (Bashar and Chakma, 2014) However, application of chemicals is not recommended because of potential toxicity for life (Akter *et al*., 2009). Some of the surveys as in Kuwait report that farm workers are very much aware of the exposure of pesticides and other related chemicals (Jallow *et al*., 2017). Conversely, Dinham report that most farmers have limited awareness about pesticides (Dinham, 2003), which is risky for both human and environmental.

In this scenario, methods like plant extracts as well as microbial biological compounds, can control infection diseases to greater extent (Mishra and Arora, 2012; Wolf *et al*., 2008). Hence, research in this area is required inhibit pathogenic diseases (Forsberg *et al*., 2002). Various studies showed that extracts of some plant can be very effective chemo therapeutants (Cabral, 2013; Pawar, 2011) which have an additional advantage of biodegradable nature (Tayel *et al*., 2015; Szopinska *et al*., 2010). One of such plants is lemongrass (*Cymbopogon citratus*) which possesses strong antimicrobial activity comparable to antibiotic of neomycin (Mandiriza *et al*., 2018). In present study antifungal activity of lemongrass extracts towards brinjal was investigated in detail.

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Lemongrass is C4 aromatic plant of Family Gramineae having 140 species (Kumari *et al*., 2007). It is native to Pakistan, India, Vietnam, Thailand, Cambodia, Indonesian Islands (Balakrishnan *et al*., 2015; Chagonda and Makanda, 2000), Africa, Australia, Europe, south America and north America (Joy *et al*., 2006) and cultivated in subtropical and tropical countries (Figueirinha *et al*., 2008). *C. citratus* is widely used as herbal tea, insect repellent and carminative (Beemnet *et al*., 2010; Kumari *et al*., 2009; Joy *et al*., 2006). Its chemical composition changes with change in habitat, genetic diversity and treatment of the culture agronomically (Carlson *et al*., 2001).

Essential oil obtained from lemongrass contains 75% (w/w) citral which is biologically more active (Huynh *et al*., 2008). Natural combination of citral is of 2 isomeric aldehydes, neral (β-citral) and isomer geranial (α-citral) (Pengelly, 2004). Lemongrass is used to cure blood circulation problem, oily skin, acne, headaches flatulence scabies (Pearson, 2010) and cancer chemo preventive (Puatanachokchai *et al*., 2002) and many more. *C. citratus* is usually used in traditional medicine for the treatment of gastro-intestinal disorders, anti-pyretic, diuretic, antispasmodic, neurological disorder, analgesic and as an antibacterial (Santin *et al*., 2009). Lemongrass shows antimicrobial activity because of the high content of citral and other essential oil constituents (Calo *et al*., 2015).

In this study, the efficacy of lemongrass leaves powder and its extracts against fungal diseases on brinjal were evaluated. Plant growth, *in vivo* and *in vitro* test showed that the extract effectively prevented the fungal diseases and enhanced the plant growth to greater extent.

Material and Method

Plant material, antagonists and pathogen. Brinjal (F1 Eggplant Advanta 305) ICI certified seeds were purchased from the market of Swabi (local) and used as test plant, while the lemongrass used in the present study as an antagonistic agent and collected from the local market of Yar Hussain (Swabi). Leaves of the plant of lemongrass were used in this study. Pathogens were isolated by soil dilution technique used for *Fusarium* species (Nash and Snyder, 1962). For the isolation of *Rhizoctonia solani* Baiting method was used (Wilhelm, 1995). *Macrophomina phaseolina* was isolated by the method prescribed by Sheikh and Ghaffar (1975).

Preparation of the plant crude extracts. Lemongrass were dried in air on bench and then powdered by using an electric grinder machine. Lemongrass powder was taken 500 g and stepwise, extractions were performed with 1 L for 500 g plant material of hexane followed by 1 L chloroform and finally by 1 L methanol (Okogun, 2000). These extracts were put on a shaker at rate of 100 rpm for 48 h and filtered. Afterwards, the solvents (hexane, chloroform and methanol) were removed by evaporation by using Rotary shaker and crude extracts of the solvents were obtained, weight was measured and kept in glass vials at temperature of 4 °C for further process.

Use of crude extract as antifungal agent. Stock solution (50 mg/mL) was prepared from solvent extract of lemongrass by dissolving it in the respective solvents. The control included respective solvents and fungicide benlate. All extracts including hexane, chloroform and methanol were used against all test fungi to find out the antifungal activity of lemongrass. Thick sterilized disc (5 mm) of Whatsman filter paper was loaded with each solvent at 10 µL, 20 µL, 30µL and 50 µL/disc and then dried. The one si de of petri dishes were inoculated with 5 mm disc of fresh and activity growing culture of test fungi like *R. solani*, *F. solani*, *F. oxysporum* and *M. phaseolina* and other sides treatments loaded discs were placed at one side of the Czapek's Dox agar (pH 7.2) petri dishes. Each treatment was replicates three times and petri plates were keep for seven days at 28 °C. The distance between disc and fungal colony was measured in milli meter and noted as zone of inhibition. Disc loaded with only was solvent considered as control.

Field experiment. A field trial was piloted in the Botanical Garden of Women University Swabi, Pakistan in randomized block pattern. The soil was invaded naturally of 0 to 5% colonization of *R. solani*, 1 to 6 sclerotia/g of soil of *M. phaseolina* and 3500 cfu/g soil of *F. solani*. Different solutions (1% w/v, 3% w/v and 5% w/v) of the leaves of lemongrass were put in sandy loam soil at 1000 mL per row and watered for two weeks after 2 to 3 days interval for the decomposition of organic matters. Afterwards, three weeks old equal sized Brinjal seedlings were planted at the edges of treated field. Each treatment was done in triplicate with 12 seedlings per row. It was watered two times in a week depending upon the conditions i.e. soil moisture and weather. Seedling planted in the untreated rows of experimental field was taken as control. After 45 days of plantation, five plants from each replicate were

uprooted and examined for the infection caused by pathogens and were subjected to further process.

Isolation of root rotting fungi. Five plants rooted out from each treatment were rinsed under tap water and sterilized with 1% bleach. Five equal root pieces size (1 cm length) were placed on potato dextrose agar (PDA) plats which were already treated with streptomycin (0.2 g/L) and penicillin (100,000 units/L). Fungi appeared after incubation period of five days at 28 °C, identified, infection and colonization percentage was calculated and weight (in g) of both root and shoot of Brinjal plant was also measured.

Statistical analysis. The data of fresh root and shoot weight, root and shoot length, infection and colonization percentages were subjected to one-way analysis of variance (ANOVA) and compared using LSD test. All analysis was performed using IBM SPSS statistical package reported by (Sokal and Rohlf, 1995).

Results and Discussion

***In-vitro* antifungal activity.** Result of antifungal activity of plant extract at different solvents (hexane, chloroform and methanol) at different concentration (10, 20, 30 and 50 µL) against test fungi i.e. *F. oxysporum, M. phaseolina*, *F. solani* and *R. solani* indicated that the best activity was shown by n-hexane solvent at 50 µL concentration against all four test fungi and produced maximum inhibition zone (Table 1).

Field experiment. The field experiment was performed to observe the efficacy of lemongrass, highly significant (P<0.0.5) root (10.38 cm) and shoot (13.2 cm) length were observed to increase at 5% w/v. Shoot weight was also increased significantly (P<0.05) as 21.244 g at 5% w/v (Fig. 1 and Table 2). Infection percent *of F. solani* was significantly (P<0.05) controlled (46.66% and 20%) at 1% and 5% w/v respectively. Infection percent of all four test fungi included. *R. solani*, *M. phaseolina*, *F. solani* and *F. oxysporum* were significantly (P<0.05) reduced with lemongrass extract when applied with

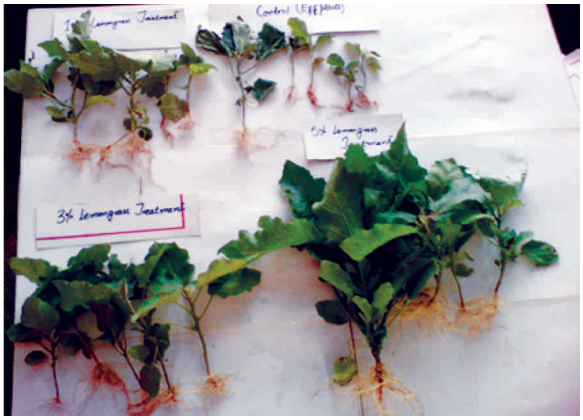

Fig. 1. Comparison of 1%, 3% & 5% w/v treatent of lemongrass with control plants of brinjal under field condition.

Table 1. Effect of ethanoic extract of lemongrass and produced zone of inhibition root infecting fungi *M. phaseolina*, *Rhizoctonia solani*, *F. solani* and *F. oxysporum*

Treatment	Concentration	*M. phaseolina* (mm)	*F. solani* (mm)	*F. oxysporum* (mm)	*R. solani* (mm)
n-Hexane	Control	0	0	0	0
	10 µL	4	2	2	4
	20 µL	6	5	4	6
	30 µL	7	6	5	8
	50 µL	10	9	10	10
Chloroform	10 µL	0	2	1	0
	20 µL	3	2	2	5
	30 µL	5	4	2	4
	50 µL	5	4	4	7
Methanol	10 µL	0	1	1	0
	20 µL	3	1	2	4
	30 µL	3	4	4	6
	50 µL	5	6	6	7
	LSD	0.765	0.745	0.798	0.915

different concentrations, while maximum reduction in infection and colonization percent of all test fungi was observed at 5% w/v shown in (Fig. 2 and Fig. 3).

Use of inorganic chemicals to control root rotting fungi and plant parasitic nematodes cause soil pollution and is also very expensive method (Bhardwaj *et al*., 2014).

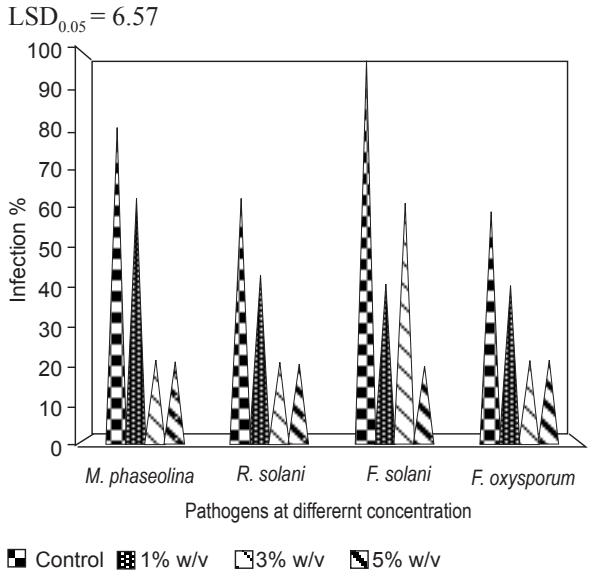

Fig. 2. Effect of lemongrass on infection percent of root infecting fungi under field condition.

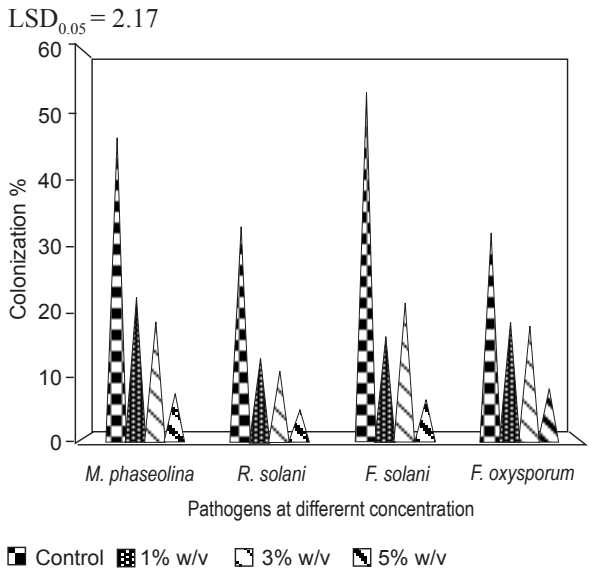

Fig. 3. Effect of lemongrass on colonization percent of root infecting fungi under field condition.

Table 2. Effect of lemongrass on different physiological parameters of brinjal plants under field condition

Treatment	Root length (cm)	Shoot length (cm)	Root weight (cm)	Shoot weight (cm)
Control	1.44	5.3	0.271	0.370
1% w/v	5.56	11.4	0.704	3.835
3% w/v	3.92	9.7	0.560	4.474
5% w/v	10.38	13.2	1.703	21.244
LSD=0.05	1.353	2.796	1.3013	13.143

Application of biocontrol agent is very effective and can be used as an alternate method in the replacement of pesticides to control the root rotting fungi (Parveen *et al*., 2019). In this work, use of organic amendment such lemongrass (*C. citratus*) reduced *F. solani*, *R. solani*, *F. oxysporum* and *M. phaseolina* infection of brinjal plant and increased growth parameters. Plant extracts are potential sources of original antimicrobial compounds (Mostafa *et al*., 2018). Similarly our study reveals that solvent fraction of n-hexane represented highly significant zone of inhibition against all test fungi (Bajpai and Kang, 2012; Al-Reza *et al*., 2010; Bajpai *et al*., 2008). The plant extracts of lemongrass inhibited fungal growth but their effectiveness varied with the concentration.

It was reported earlier that eggplant is prone to several pest and insects particularly *Fusarium oxysporum F. melongenae*) and root knot nematodes *Meloidogyne* spp. resulting in lower yield (Phap *et al*., 2010). During the field experiment it was seen that soil modification with *C. citratus* presented suppressive effect to appreciable extent on the pathogens and hence enhanced growth of Brinjal (*S. melongena*). Parveen *et al*. (2019) reported that organic amendments showed better results and create a friendly environment without any pollution, not only enhancing the growth but also reducing the infection of root rotting fungi.

Conclusion

In the present study, extracts of fresh leaf *C. citratus* was found as an efficient biological extract for controlling different fungi like, *F. solani*, *F. oxysporum*, *M. phaseolina* and *R. solani*. The application of these biological extracts is cheap, environmental friendly and is potentially useful for small scale farmers as it is locally available. Moreover, when lemongrass was used as organic amendment for the growth enhancement of

brinjal plant, it also showed positive affect on growth enhancement of brinjal plant.

Conflict of Interest. The authors declare no conflict of interest.

References

Akter, S., Brouwer, R., Brander, L., Beukering, P. 2009. Respondent uncertainty in a contingent market for carbon offsets. *Ecological Economics*, **68:** 1858-1863. https://doi.org/10.1016/j.ecolecon.2008.12.013

Al-Reza, S.M., Rahman, A., Ahmed, Y., Kang, S.C. 2010. Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum* L. *Pesticide Biochemistry and Physiology*, **96:** 86-92. DOI:10.1016/j.pestbp.2009.09.005

Altinok, H.H., Can, C. 2010. Characterization of *F. oxysporum F. melongenae* isolates from eggplant in Turkey by pathogenicity, VCG and RAPD analysis. *Phytoparascitica*, **38:** 149-157. DOI:10.1007/s12600-010-0081-0

Bajpai, V.K., Kang, S.C. 2012. *In vitro* and *in vivo* inhibition of plant pathogenic fungi by essential oil and extracts of *Magnolia liliflora* Desr. *Journal of Agricultural Science and Technology*. **14:** 845-856. URL: http://jast.modares.ac.ir/article-23-543-en.html

Bajpai, V.K., Shukla, S., Kang, S.C. 2008. Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L. *Bioresource Technology*, **99:** 8903-8908. https://doi.org/10.1016/j.biortech.2008.04.060

Balakrishnan, A., Priya, V., Gayathri, R. 2015. Preliminary phytochemical analysis and antioxidant activities of lemongrass and lavender. *Journal of Pharmaceutical Research*, **7:** 448-450. https://www.jpsr.pharmainfo.in/Documents/Volumes/vol7Issue07/jpsr07071514.pdf

Bashar, M.A., Chakma, M. 2014. *In vitro* control of *F. solani* and *F. oxysporum* the causative agent of brinjal wilt. *Dhaka University Journal of Biological Science*, **23:** 53-60. https://doi.org/10.3329/dujbs.v23i1.19826

Beemnet, M.K., Omarsherif, M., Tsion, T., Solomon, A.M. 2010. *Production*, *Processing & Utilization of Aromatic Plants*, 31 pp., Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa, Ethiopia. https://doi.org/10.5897/AJAR2019.13984

Bhardwaj, D., Ansari, M.W., Sahoo, R.K., Tuteja, N. 2014. Biofertilizer function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, **13:** 66. https://doi.org/10.1186/1475-2859-13-66

Biswas, R., Banerjee, A., Halder, U., Bandopadhyay, R. 2018. Transgenic Research in Vegetable Crops with Special Reference to Brinjal. In: *Genetic Engineering in Horticultural Crops*, **Chapter 8,** pp. 155-167, Elsevier Inc. https://doi.org/10.1016/B978-0-12-810439-2.00008-8

Cabral, L., Cruz, D., Fernandez, V., Patriarca, P.A. 2013. Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. *International Journal of Food Microbiology*, **166:** 1-14. https://doi.org/10.1016/j.ijfoodmicro.2013.05.026

Calo, J.R., Crandall, P.G., O'Bryan, C.A., Ricke, S.C. 2015. Essential oils as antimicrobials in food system. *Food Control*, **54:** 111-119. https://doi.org/10.1016/j.foodcont.2014.12.040

Carlson, L.H.C., Machado, R.A.F., Spricigo, C.B., Pereira, L.K., Bolzan, A. 2001. Extraction of lemongrass essential oil with dense carbon dioxide. *Journal of Supercritical Fluids*, **21:** 33-39. DOI: 10.1016/S0896-8446(01)00085-7

Chagonda, L.S., Makanda, C. 2000. Essential oils of cultivated *Cymbopogon winterianus* (Jowitt) and of *Cymbopogon citratus* (DC) (Stapf) from Zimbabwe. *Journal of Essential Oil Research*, **12:** 478-480. https://doi.org/10.1080/10412905.2000.9699570

Chellemi, D.O. 2002. Non chemical management of soil borne pests in fresh market vegetable production systems. *Phytopathology*, **92:** 1367-1372. https://doi.org/10.1094/PHYTO.2002.92.12.1367

Dinham, B., Malik, S. 2003. Pesticides and human rights. *International Journal of Occupational and Environmental Health*, **9:** 40-52. https://www.tandfonline.com/doi/abs/10.1179/107735203800328867

FAO FAOSTAT data. 2012. (Available at: http://www.fao.org Retrieved on 25 February, 2014).

FAO. Food and Agricultural Organization of the United Nations. 2014. The state of the world's land and water resources for food and agriculture. Available at http://www.fao.org/docrep/017/i1688e/i1688e.pdf.

Figueirinha, A., Paranhos, A., Perez-Alonso, J.J., Santos-

Buelga, C., Batista, M.T. 2008. *Cymbopogon citratus* leaves. Characterization of flavonoids by HPLC-PDA-ESI/MS & an approach to their potential as a source of bioactive polyphenols. *Food Chemistry*, **110:** 718-728. DOI: 10.1016/j.foodchem.2008.02.045

Forsberg, G., Hernell, O., Melgar, S., Israelsson, A., Hammarström, S., Hammarström, M.L. 2002. Paradoxical co-expression of pro-inflammatory and down regulatory cytokines in intestinal T cells in childhood celiac disease. *Gastroenterology*, **123:** 667-678. https://doi.org/10.1053/gast.2002.35355

Huynh, K.P., Maridable, J., Gaspillo, P., Hasika, M., Malaluan, R., Kawasaki, J. 2008. Essential oil from lemongrass extracted by supercritical carbon dioxide and steam distillation. *Phillippine Agricultural Scientist*, **91:** 36-41. https://agris.fao.org/agris-search/search.do?recordID=PH2009000163

Jallow, M.F.A., Awadh, D.G., Albaho, M.S., Devi, V.Y., Thomas, B.M. 2017. Pesticide knowledge and safety practices among farm workers in Kuwait: results of a survey. *International Journal of Environmental Research and Public Health*, **14:** 1-15. doi: 10.3390/ijerph14040340.

Joy, P.P., Baby, P.S., Samuel, M., Gracy, M., Ancy, J. 2006. Lemongrass: the fame of Cochin. *Indian Journal of Arecanut Species and Medicinal Plants*, **8:** 55-64. https://www.scribd.com/document/416502165/LemongrassFAMEOFCOCHIN-pdf

Kashyap, V., Vinod, S., Collonnier, K.C., Fusari, F., Haicour, R., Rotino, G.L., Sihachakr, D., Rajam, M.V. 2003. Biotecnology of eggplant. *Scientia Horticulturae*, **97:** 1-25. https://doi.org/10.1016/S0304-4238(02)00140-1

Kudlu, C., Stone, G.D. 2013. The trials of genetically modified food. *Food*, *Culture and Society*, **16:** 21-42. https://doi.org/10.2752/175174413X13500468045326

Kumar, V., Chopra, A.K. 2016. Agronomical performance of high yielding cultivar of eggplant (*Solanum melongena* L.) grown in sewage sludge amended soil. *Research in Agriculture*, **1:** 1-24. http://www.scholink.org/ojs/index.php/ra

Kumar, V. 2015. Effects of treated sugar mill effluent irrigation on soil and hybrid cultivar of eggplant (*Solanum melongena* L.) under field conditions. *Journal of Environmental Health Science*, **1:** 1-11. DOI: 10.15436/2378-6841.15.021

Kumari, J., Verma, V., Goyal, A., Shahi, A.K., Sparoo, R., Sangwan, R.S., Qazi, G.N. 2009. Genetic diversity analysis in *Cymbopogon* species using DNA markers. *Plant Omics*, **2:** 20-29. http://www.pomics.com./Akash_KumarJanuary2009_2_1_20_29.pdf

Kumari, R., Srivastava, M., Dubey, N.K. 2007. Evaluation of *Cymbopogon martini* oil extract for control of postharvest insect deterioration in cereals and legumes. *Journal of Food Protection*, **70:** 172-178. DOI: 10.4315/0362-028X-70.1.172

Mandiriza, G., Kritzinger, Q., Aveling, T.A.S. 2018. The evalution of plant extracts, biocontrol agent and hot water as seed treatments to control black rot of rape in south Africa. *Crop Protection*, **114:** 129-136. https://doi.org/10.1016/j.cropro.2018.08.025

Mishra, S., Arora, N.K. 2012. Evaluation of rhizospheric *Pseudomonas* and *Bacillus* as biocontrol tool for *Xanthomonas campestris* pv campestris. *World Journal of Microbiology and Biotechnology*, **28:** 693-702. doi: 10.1007/s11274-011-0865-5

Mostafa, A.A., Al-Askar, A.A., Almaary, K.S., Dawoud, T.M., Sholkamy, E.N., Bakri, M.M. 2018. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Science*, **25:** 361-366. doi: 10.1016/j.sjbs.2017.02.004. Epub 2017 Feb 24.

Nash, S.M., Snyder, W.C. 1962. Quantitative estimations by plate counts of propagules of the Bean root rot Fusarium in field soils. **52:** 567-572. http:// garfield.library.upenn.edu/classics1982/A1982NZ582000001.pdf

Okogun, J.I. 2000. Methods of medicinal plant extract preparation. *National Institute for Pharmaceutical Research and Development (NIPRD)*, Idu–Abuja, Nigeria.

Parveen, G., Mukhtar, N., Kaleemullah, Muhammad, S.K. 2019. Enhancement of growth and suppressing the root diseases of tomato plant by using organic amendment. *Pure and Applied Biology*, **8:** 1092-1099. https://thepab.org/index.php/journal/article/view/878

Pawar, V.K., Kulkarni, G.T., Awasthi, R. 2011. Phytosomes: an approach to increase the bioavailability of plant extracts. *International Journal of Pharmaceutical Science*, **3:** 1-3. https://www.researchgate.net/publication/230727931_Phytosomes_An_Approach_to_Increase_The_Bioavailability_of_Plant_Extracts

Pearson, O. 2010. The antibacterial properties of

Essential oils. http://www.livestrong.com/article/168697-the-antibacterialproperties-of-essential-oils/# ixzz1W7u9hn6K.

Pengelly, A. 2004. The constituents of medicinal plants (eds): *An Introduction to the Chemistry and Therapeutics of Herbal Medicine*, pp. 85-103, CABI Publishing, United Kingdom. DOI: 10.4324/9781003117964

Phap, P.D., Xuan, H.T.L., Sudhakar, D., Subramanian, P. 2010. Engineering resistance in brinjal against nematode (*Meloidogyne incognita*) using *cry1Ab* gene from *Bacillus thuringiensis* Berliner. In: *3rd International Conference Development Biomedical Engineering in Vietnam*, pp. 278-281, Ho Chi Minh City, Vietnam. https://link.springer.com/chapter/10.1007/978-3-642-12020-6_70

Prodhan, M.Z.H., Hasan, M.T., Chowdhury, M.M.I., Alam, M.S., Rahman, M.L., Azad, A.K., Hossain, M.J., Naranjo, S.E., Shelton, A.M. 2018. Bt eggplant (*Solanum melongena* L.) in Bangladesh: fruit production and control of eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenee), effects on non-target arthropods and economic returns. *Public Library of Science*, **13:** 1-17. https://doi.org/10.1371/journal.pone.0205713

Puatanachokchai, R., Kishida, H., Denda, A., Murata, N., Konishi, Y., Vinitketkumnuen, U., Nakae, D. 2002. Inhibitory effects of lemongrass (*Cymbopogon citratus*, Stapf) extract on the early phase of hepatocarcinogenesis after initiation with diethyl nitrosamine in male Fischer 344 rats. *Cancer Letters*, **183:** 9-15. https://doi.org/10.1016/S0304-3835(02)00111-8

Santin, M.R., Santos, A.O., Nakamura, C.V., Ferrira, I.V., Ueda-Nakamura, T. 2009. *In vitro* activity of the essential oil of *Cymbopogon citratus* and its major component (citral) on *Leishmania Amazonensis*. *Parasitology*, **5:** 1489-1496. doi: 10.1007/s00436-009-1578-7.

Sheikh, A.H., Ghaffar, A. 1975. Population study of Sclerotia of *Macrophomina phaseolina* in cotton field. *Pakistan Journal of Botany*, **7:** 13-17. https://agris.fao.org/agris-search/search.do? recordID=US201303022610

Sokal, R.R., Rohlf, F.J. 1995. *Biometry: The Principles and Practices of Statistics in Biological Research*, 887 pp., Freeman, New York, USA.

Szopinska, D., Jensen, B., Knudesen, M.B., Tylkowska, K., Dorna, H. 2010. Non-chemical methods for controlling seedborne fungi in carrot with special reference to *Alternaria radicina*. *Journal of Plant Protection Research*, **50:** 184-192. DOI: 10.2478/v10045-010-0031-3

Tayel, A.A., Moussa, S.H., Salem, M.F., Mazrou, K.E., El-Tras, W.F. 2015. Control of citrus molds using bioactive coatings incorporated with fungal chitosan/plant extracts composite. *Journal of the Science of Food and Agriculture*, **96:** 1306-1312. https://doi.org/10.1002/jsfa.7223

Wilhelm, S. 1955. Longevity of *Verticilliuim* wilt fungus in laboratory and field. *Phytopathology*, **45:** 180-181. https://www.cabdirect.org/cabdirect/abstract/19551102133

Wolf, J.M., Van der Birnbaum, Y.E., Zouwen, P.S., van der Groot, S.P.C. 2008. Disinfection of vegetable seed by treatment with essential oils, organic acids and plant extract. *Seed Science and Technology*, **36:** 76-88. https://doi.org/10.15258/sst.2008.36.1.08