Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.043

MANAGEMENT OF *FUSARIUM* SPECIES ISOLATED FROM *ZINGIBER OFFICINALE* (GINGER) BY ESSENTIAL OILS

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(Date of Receiving-06-10-2020; Date of Acceptance-03-01-2021)

ABSTRACT

Mycological examination of 57 samples of Ginger revealed the occurrence of toxigenic *Fusarium* species (*Fusarium verticililoides, F. oxysporum, F. solani and F. equiseti*) with 44.9% of incidence. *Fusarium* species isolated in this study were treated with different concentration (1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm) of five essential oils viz., Clove, Eucalyptus, Cedar wood , Peppermint and Lemon grass and the antifungal activity of each essential oils was evaluated. The results indicated that all essential oils exhibited anti-fungal activity at different concentrations. Peppermint oil was highly effective in inhibiting all the *Fusarium* species tested at 1500ppm and higher concentration. Lemongrass, Clove and Eucalyptus oils inhibited the fungal growth at 2000-2500 ppm. Cedar oil was less effective in inhibition of mycelial growth and sporulation of the *Fusarium* species tested. The present results reveals that essential oils can be used as natural fungicide at lowest concentrations for controlling *Fusarium* species in Ginger

Keywords: *Fusarium verticillioides*, antifungal activity, essential oil, mycotoxins

INTRODUCTION

Ginger (*Zingiber officinale*) is a herbaceous perennial, grown for its aromatic rhizomes, used as a spice and medicine (Ramteke and Kamble, 2011). They play an important role in human nutrition and health. Pharmaceutical, perfumery and cosmetic industry also makes extensive use of these plant products. *Zingiber ofiicinale* is vulnerable to microbial contaminaton during pre and post-harvest handling. Fungal species belonging to *Fusarium, Aspergillus* and *Penicillium* are majorly responsible for chemical, nutritional changes and mycotoxin contamination (Adebayo-Tayo *et al.,* 2012). Mycotoxigenic fungi infected to agricultural commodities can lead to reduced quality, yield reduction and mycotoxicosis among humans and livestock. The management of mycotoxigenic fungi and their subsequent mycotoxins is necessary for sustainable, safe food and feed production. The major problem in many countries is the reduction of mycotoxin contamination in agricultural commodities and various preventive measures also have been established for the same in a variety of settings: before harvest and after harvest (in storage, transportation, or processing). All pre-harvest strategies aim to avoid the development mycotoxins and post-harvest practices target to detoxify mycotoxin produced (Luo *et al.,* 2018).

Synthetic fungicides and chemical preservatives play a significant role in controlling mycotoxigenic fungi , but studies on their toxicity indicates that they cause adverse effect on humans, animals and non-target organisms (Mesnage *et al.,* 2014) and negative impact on environment (da Cruz *et al.,* 2013). These factors have resulted in increased search for alternatives to conventional fungicides for effective management of mycotoxigenic fungi (Gakuubi *et al.,* 2017). Non-chemical processes based on plant extracts and secondary metabolites including essential oils, flavonoids, phenols, tannins, alkaloids, quinons, saponins and sterols for the treatment of microbial infection have played an important role in inhibiting pathogen growth and thus improving food quality (Mossini *et al.,* 2004; Amini *et al.,* 2012; Anjorin *et al.,* 2013). Essential oils are a rich source of biologically active compounds and they are potential sources of novel antimicrobial compounds (Adjou *et al.,* 2012). Many scientific studies have shown that essential oils obtained from various parts of plants have antimicrobial activity against many fungal pathogens. They can be used in integrated pest management programs as they are biodegradable compounds and are classified as "generally recognized as safe" (GRAS) by Food and Drug Administration (FDA) (Perczak *et al.,* 2019a).

In the present study *Ginger* samples were collected from various districts of Karnataka state, India and subjected to mycological examination. *Fusarium species* isolated from Ginger were tested with different concentrations of essential oils and observed for inhibition of mycelial growth. This study has pivotal role in determining and controlling the mycotoxigenic fungal isolates associated with ginger and ginger derived products.

MATERIALS AND METHODS

Sampling area: Fifty seven (57) Ginger samples were collected from southern districts of Karnataka state, India

Sources of Essential Oils: Essential oils

Table-1: Statistical analysis of Essential oils by t-test.

Essential Oils	#Conc in ppm	F.vert colony (cm)	F.oxy colony (cm)	F.sol colony (cm)	F.equi colony (cm)
	Control	6.0±0.17	6.5±0.06	5.2±0.25	6.7±0.21
	1000	5.8±0.06^{NS}	4.9±0.06^{c}	2.5±0.06^{c}	5.2±0.06^{c}
Clove	1500	5.2±0.15^{b}	1.0±0.00^{c}	2.4±0.26^{c}	1.5±0.06^{c}
	2000	2.2±0.15^{c}	0.5±0.00^{c}	1.1±0.06^{c}	0.6±0.06^{c}
	2500	0.6±0.15^{c}	0.5±0.00^{c}	1.0±0.06^{c}	0.5±0.06^{c}
	Control	5.8±0.25	6.5±0.06	5.2±0.25	6.6±0.38
	1000	1.8±0.10^{c}	2.7±0.12^{c}	1.8±0.00^{c}	1.8±0.15^{c}
Eucalyptus	1500	1.4±0.17^{c}	1.1±0.10^{c}	1.6±0.23^{c}	1.0±0.06^{c}
	2000	1.2±0.25^{c}	1.1±0.15^{c}	1.5±0.17^{c}	0.5±0.06^{c}
	2500	1.0±0.00^{c}	0.9±0.10^{c}	0.9±0.06^{c}	0.5±0.00^{c}
	Control	6.5±0.40	6.5±0.06	5.7±0.10	6.6±0.38
	1000	6.2±0.10^{NS}	6.3±0.15^{NS}	5.5±0.06^{a}	5.3±0.32^{a}
Cedar	1500	5.0±0.10^{c}	6.0±0.10^{c}	5.1±0.10^{b}	4.3±0.12^{c}
	2000	3.6±0.20^{c}	5.0±0.10^{c}	4.6±0.15^{c}	4.1±0.15^{c}
	2500	2.7±0.12^{c}	3.4±0.06^{c}	4.3±0.25^{c}	3.4±0.10^{c}
	Control	5.8±0.10	6.6±0.06	5.7±0.26	7.4±0.20
	1000	2.5±0.12^{c}	2.5±0.12^{c}	4.8±0.15^{b}	1.9±0.06^{c}
Lemongrass	1500	2.1±0.10^{c}	2.1±0.10^{c}	2.9±0.15^{c}	1.2±0.06^{c}
	2000	0.7±0.06^{c}	0.7±0.06^{c}	1.6±0.06^{c}	0.5±0.00^{c}
	2500	0.5±0.00^{c}	0.5±0.00^{c}	1.2±0.10^{c}	0.5±0.00^{c}
	Control	5.8±0.10	6.6±0.06	5.7±0.26	7.4±0.20
	1000	1.1±0.10^{c}	3.6±0.06^{c}	1.5±0.12^{c}	4.6±0.10^{c}
Peppermint	1500	0.5±0.00^{c}	1.5±0.10^{c}	1.2±0.12^{c}	0.5±0.06^{c}
	2000	0.5±0.00^{c}	0.5±0.00^{c}	0.5±0.00^{c}	0.5±0.00^{c}
	2500	0.5±0.00^{c}	0.5±0.00^{c}	0.5±0.00^{c}	0.5±0.00^{c}

^{NS}; not significant, ^{a} $p<0.05$, ^{b} $p<0.005$, ^{c}$p<0.0005$

Abbreviations: #Conc- Concentration, *F.vert- Fusarium verticillioides, F.oxy- Fusarium oxysporum, F.sol- Fusarium solani, F. equi- Fusarium equiseti.*

(Eucalyptus, clove, lemon grass and peppermint) were collected at Natural and essential oils limited, Mysore, Karnataka. Cedar wood oil was purchased from Karnataka Aromas, Bangalore, India.

Test Isolates: *Fusarium* species viz., *Fusarium verticillioides*, *F. oxysporum, F.equiseti F. solani* were isolated from Ginger samples by agar plate method and blotter method. The isolated *Fusarium* colonies were pure cultured and identified based on micro morphological characteristics using fungal keys and manuals (Leslie and Summerell, 2006). *Fusarium* isolates were confirmed PCR amplification using universal ITS primers.

Determination of antifungal activity of essential oils: Antifungal activity of each essential oil was determined by Poisoned food technique (Gakuubi *et al.,* 2017). Four different concentrations of five essential oils eucalyptus, clove, lemongrass, peppermint, cedar oils was prepared by incorporating 20ml of PDA medium with requisite quantity of each oil separately to prepare concentrations of 1000, 1500, 2000 and 2500 ppm 0.05% (v/v) Tween 80 was added

as an emulsifying agent. Agar disk (0.5 cm) was taken from periphery of five-day-old culture of each *Fusarium* isolate and inoculated on to the centre of the agar plate and incubated at 25 ± 2^0C light and dark for 12 hours each up to 5 days. Three replicates were maintained for each treatment and control set consisted of PDA fungal culture medium without adding any essential oils. The inhibitory effect was evaluated by measuring fungal colony diameter with the help of centimeter scale and compared with control. The diameter of the initial disk was recorded as (0.5cm) in case of full inhibition of fungal colony and it was considered as Minimum inhibitory concentration (MIC) (Sreenivasa *et al*, 2011).

Statistical analysis: Statistical interpretation of the results was made using t - test and the differences between concentration used and *Fusarium* species tested was determined by three way ANOVA test (oil v/s concentrations v/s organism). Tukey's multiple comparison tests (p = 0.05) was applied in case of a significant impact to identify the effectiveness of various concentrations against fungal species.

RESULTS AND DISCUSSION

The efficacy of five essential oils tested against isolated *Fusarium* species namely *F. verticillioides, F. solani, F. oxysporum,* and *F. equiseti* and statistical analysis was performed (Table 1 and 2). T-test analysis was carried out by comparing control with essential oil treated fungi and the results revealed that all the *Fusarium* species were significantly reduced by Peppermint, Eucalyptus and Lemongrass oils compared to Clove and Cedar oils (Figure:1). Inhibitory effect of essential oils was directly proportional to the concentration used except for Cedar oil. Minimum inhibitory concentration (MIC) for *F. verticillioides* was found to be 2500ppm for Clove and Eucalyptus, 2000 ppm for Lemongrass and 1500ppm for Peppermint oil. MIC for *F. oxysporum* was 2000ppm for Clove, Lemongrass and Peppermint oils, 2500 ppm for Eucalyptus oil. MIC for *F. solani* was observed at 2500 ppm and 2500 ppm using Eucalyptus and Peppermint oil respectively, where as other essential oils did not show significant inhibition. MIC for *F. equiseti* was recorded at 2000 ppm using Lemongrass, Clove, Eucalyptus and 1500 ppm with Peppermint oil. Cedar oil was not effective in inhibiting the mycelial growth irrespective of oil concentrations.

Multiple comparison analysis using Tukey's test revealed that all the essential oils tested were effective in reducing mycelial growth and spore germination at various concentrations. At lower concentrations of 1000ppm Eucalyptus oil reduced the growth of *F. verticillioides* and *F.solani* compared to other species whereas Clove oil was effective in reducing the mycelial growth at 2000 and 2500ppm for *F. oxysporum* and *F. equiseti*. The inhibitory effect of Lemon grass was observed for *F. verticillioides , F.oxysporum* and *F. equiseti.* The Peppermint oil was more effective for *F. verticillioides* and *F.equiseti* at various concentrations .Cedar oil was less effective in reducing the mycelial growth compared to other oils but did not inhibit the fungal growth completely.

Lemongrass, Eucalyptus and Peppermint oils were found to be very effective in inhibiting all of the *Fusarium* species tested at different concentrations and this complies with the earlier findings of the research group who pointed out that essential oils from citronella, peppermint, lemongrass, and clove are effective against many *Fusarium* species (Sreenivasa *et al.,* 2011). The inhibitory potential of Clove, Lemongrass and Eucalyptus against *F. oxysporum* was recorded at very lower concentrations from 125 ppm-500ppm (Sharma *et al.,* 2017). Among seventy five essential oils screened, *F. oxysporum was* sensitive to most of the essential oils. Lemongrass was most effective and Clove was less effective to *F. oxysporum* (Pawar and Thaker, 2007) .In contrast, studies conducted elsewhere reported that Clove has antimicrobial and antifungal properties against *Fusarium* species (Massoud *et al.,* 2012; Deng *et al.,* 2013; Thabet and Khalifa ,2018). *Fusarium solani* and *F. oxysporum* can be inhibited by leaf extracts and essential oils of Eucalyptus respectively (Bashir and Tahira, 2012; Regmi and Jha, 2018). Inhibition of 98% of spore germination of *F.verticillioides, F.oxysporum* and *F.solani* by Eucalyptus oil was observed at 500ppm (Elgorban *et al.,* 2015). The effects of twelve essential oils on *F.oxysporum* and compared with commercial fungicides was examined and found that fungicide Prosaro -250 EC and essential oils from clove, fennel, anise, true lavender, peppermint, thyme, basil , tea and sage exhibited the same inhibitory effect (Palfi *et al* ., 2019). The inhibition of *F.graminearum and F. culmorum* in maize and wheat seeds and reduction in the Deoxynivalenol, zearalenone and group B trichothecenes level with the application of essential oils was reported (Perczak *et al.,* 2019a ; 2019b). Kumar *et al*. (2016) reported the inhibition of growth and mycotoxin (ZEA) production by *Fusarium graminearum* at 3.5 and 3 mg/mL respectively by turmeric essential oil. Another study revealed that wild oregano and garlic essential oils completely prevented the growth of *Penicillium verrucosum* and its mycotoxin production, while mint and sage essential oils significantly reduced the OTA production by *Penicillium verrucosum* (Ozcakmak *et al* ., 2017).

The present work reveals that Ginger has the potential to harbor toxigenic *Fusarium verticillioides* in significant numbers, which is capable of producing mycotoxin which causes many health hazards to humans. Ginger is consumed raw and used in ayurvedic medicine which causes adverse effects on consumer's health, hence appropriate control measures must be taken to avoid fungal contamination. This can be made possible by using biologically safe natural fungicides like essential oils. In our study all the tested essential oils were capable of reducing and inhibiting the growth of *F.verticillioides, F.oxysporum*, *F.solani and F .equiseti* at various concentrations and the results were completely dependent upon the concentration and type of essential oil used. Peppermint, Eucalyptus , Lemongrass, and Clove oils exhibited antifungal activity

Table-2 Statistical analysis by Tukey's multiple comparison test.

Essential Oils	#Conc in ppm	F ver vs F oxy	F ver vs F sol	F ver vs F equ	F oxy vs F sol	F oxy vs F equ	F sol vs F equ
Clove	1000	**	**	**	**	**	**
	1500	**	**	**	**	*	**
	2000	**	**	**	**	NS	**
	2500	NS	**	NS	**	NS	**
Eucalyptus	1000	**	NS	NS	**	**	NS
	1500	NS	NS	NS	*	NS	**
	2000	NS	NS	**	NS	*	**
	2500	NS	NS	**	NS	**	**
Cedar	1000	NS	**	**	**	**	NS
	1500	**	NS	**	**	**	**
	2000	**	**	*	NS	**	*
	2500	**	**	**	**	NS	**
Lemongrass	1000	NS	**	**	**	**	**
	1500	NS	**	**	**	**	**
	2000	NS	**	*	**	*	**
	2500	NS	**	NS	**	NS	**
Peppermint	1000	**	**	**	**	**	**
	1500	**	**	NS	*	**	**
	2000	NS	NS	NS	NS	NS	NS
	2500	NS	NS	NS	NS	NS	NS

NS; not significant, * $p<0.05$, ** $p<0.01$

Abbreviations: #Conc-Concentration, *F.ver- F.verticillioides, F.oxy-F. oxysporum, F. sol-F.solani, F. equ- F. equiseti*

hence use of these oils can increase the shelf life of ginger and can overcome losses caused during storage conditions. MIC reported as low as 1000ppm indicates the economy of essential oils as the fungal growth is inhibited in minute concentrations, economic burden is reduced. Essential oils as natural fungicide plays significant role over biological and environmental complications caused by synthetic fungicides. Therefore these essential oils are superior and ecofriendly, in view of this the authors urge concerned authority to implement laws and perpetuate the information among farmers and store keepers to apply essential oils to Ginger, a highly perishable commodity thus avoiding economical as well as health hazards.

ACKNOWLEDGEMENT

First author acknowledges the research support provided by Department of Microbiology, Yuvaraja's College and Manasagangothri, University of Mysore, Karnataka, India.

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