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Research Article

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EFFICACY OF ESSENTIAL OILS AGAINST *Penecillium sp.* CAUSING MUSHROOM MOLD UNDER *IN VITRO* CONDITION

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ABSTRACT

Mushrooms are nutritive and medicinal foods; Oyster and button are the very important mushrooms being grown at various farms, localities etc. Varieties of fungal contaminants limit the mycelium growth of the button mushroom as well as others in the substrate and affecting its yield. In this study, it was determined the *Penecillium* sp is the major mold affecting the yield of the Oyster mushroom. Furthermore *In-Vitro* experiment was carried out for management of mushroom against *Penecillium* sp. Essential oils viz., cinnamon oil, coconut oil, Neem oil and rose oil were used at various concentrations (4%, 8%, 12% and 16%) against *Penecillium sp.* All the essential oils showed impressive results, among all treatments Neem oil showed high reduction of colony growth of *Penecillium sp.* followed by coconut oil, Rose oil and Cinnamon oil. All the data was statically analyzed.

KeyWords: *In vitro* management, essential oils, Mushroom, *Penecillium* sp.

INTRODUCTION

Mushrooms are fleshy, spore-bearing fruiting body of a kind of fungus. There are vast variety of mushroom with various qualities; some are edible, which is a rich, low-calorie source of fiber, protein and anti-oxidants. Mushrooms are on the top concerning taste, smell, proteins and medicinal qualities in food items. They have potential anti-inflammatory, hyperglycemic and hypo cholesterol emic effects. Mushroom cultivation gives proper growth and high yield only when it is maintained with optimum conditions with proper care and suitable substrate. (Rosario, *et al*., 2021). There are various environmental conditions needed in the cultivation of various species of mushrooms. In the mushroom industry, several species of mushrooms are being cultivated for commercial purposes, including oyster mushrooms (*Pleurotus spp*), which are sold in markets and easily cultivated in the lowlands, while Shiitake (*Lentinus endodes*) and button (*Agaricus spp*) are cultivated in the highlands and other medicinal mushroom with high commercial value. They grow at moderate temperatures ranging from 20–35 ℃ and a humidity of 65–70 % for 6–8 months in a year. The different growing stage of each type of oyster mushroom requires different optimal temperature for most optimal growth (Haimid *et al*., 2013). Most popular edible mushrooms of the world are Cremini mushroom, Morel mushroom, Shiitake mushroom, Oyster mushroom, White button mushroom, and Straw mushroom (Joseph, 2021). Economically mushroom farming is being done in more than 100 countries of the world (Gupta *et al*., 2018). Mushrooms are fungi with significant nutritional value currently counting around 2000 edible species distributed around the world (Rathore *et al*., 2019). The most commonly cultivated basidiomycetes worldwide and in Serbia are button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus sp*.) and shiitake (Lentinus edodes). Over the past two decades, green mould caused by *T. aggressivum* has been the most serious disease of button mushroom. Fungal diseases commonly occurring in white button mushrooms include dry bubble (*Verticillium spp*.), cobweb (*Cladobotryumspp*.), green mould in compost (*Trichoderma harzianum*) and green mould on casing (*Trichoderma viride*). Over the past two decades, moulds has been the most serious diseases of mushroom and causing heavy losses (Gupta *et al*., 2018). Most of these contaminant microorganisms come from poorly sterilized substrates also. Several sterilization techniques can be employed to eliminate pre-existing contaminant microorganisms. Besides, biological control involving botanicals/ essential oils and live antagonists can also be used against microorganisms (Ghimire, *et al*., 2021).

MATERIAL AND METHODS

The experiments was conducted at laboratory department of Plant Pathology Balochistan Agriculture College, Quetta

Obtaining mushroom culture: Mushroom Spawns of Oyster were obtained from marketplace at Quetta. For the isolation of microorganism Culture media

were prepared i.e., Potato Dextrose Agar (PDA) and distilled water (DW) as given in Table 1

Table. 1. Preparation of PDA with addition of malt extract

Ingredients	g/L DW	g/0.5L DW
Dextrose	20	10
Potato Extract	4	2
Agar	15	7.5
Malt Extract	6	3
PH	7.3 ± 2 at 25-26°C	

Isolation and Morphological identification of fungi causing mold in mushroom: Mushroom substrate such as wheat grain, sorghum, millet which were prepared and mushrooms spawning was done in wheat straw, saw dust, rice brain and compost in polythene bag, tray etc. Fungal pathogen was observed such as mold in bag cultures. Isolation of fungal pathogens was done on PDA media. Isolation of Pathogen from infected substrates and spawn for growth including wheat straw, compost, spawn, upon contamination in these substrates we picked contaminated portions for further culturing. Prepared PDA media and glassware were autoclaved at 121°C for 20 minutes. The laminar flow cabinet disinfected with ethanol-soaked cotton. Then switched on the UV light inside the laminar to disinfect microbes and keep materials under UV for up to 15 minutes. The media was allowed to cool and poured onto Petri plates and it was allowed for solidification, and then transferred single colony inoculated through sterile loop in the laminar flow cabinet. The Petri plates were sealed with Para film to protect media from other contaminants. After inoculation, plates were incubated in incubator at 27°C for the growth of pathogen. Then incubated Petri dishes were observed for growing fungal contaminants. For microscopic identification, a slide with one drop of water was prepared placed the fungal pathogen through loop and rubber and kept cover slip on the slide and fixed it. Slide was kept under stage microscope and set the lens of microscope. Those lenses were 20x, 40x, 60x and 100x. Connected laptop to the microscope on the same line and measured the spore size.

Pathogenicity Test: In this experiment the substrate was subjected to treatment of polypropylene bags contain 500 g of wet substrate with the addition 1% w/w (weight by weight) of calcium carbonate (CaCo3). After they were spray with 3 ml water suspension of conidia of fungal pathogen per bags and mixed. The contamination was done with three replications per bag. Then bags were inoculated with 10 % w/w spawn of mushroom then mixed these substrate bags incubate at room temperature for 10 days and substrate bags were observed. Focused on the evaluation of the growth of the Pathogen after the hot water treatment. Two strains and three different sterilized and non-sterilized substrates were used for example Pathogen spray inoculum and mushroom spawn and in control no inoculation were performed, bags were observed after 15 days of incubation at room temperature in the dark place. (Colavolpe *et al*., 2014) was followed.

Preparation of Essential oils: The Preparation 4%, 8% 12% 16% 0% respectively essential oil prepared the solution in 20 ml Backer 4 percent concentration of essential oil 0.20ml mix ethanol 4.80ml Tween20 0.25ml and distal water (DH2O) 4.75ml the total solution 10ml 8 percent concentration of essential oil 0.40ml mix ethanol 4.60ml Tween20 0.25ml and DH2O 4.75ml the total solution 10ml 12 percent concentration of essential oil 0.60ml mix ethanol 4.40ml Tween20 0.25ml and DH2O 4.75ml the total solution 10ml 16 percent concentration of essential oil 0.80ml mix ethanol 4.20ml Tween20 0.25ml and DH2O 4.75ml the total solution 10ml 0 percent concentration of essential oil 0.00ml mix ethanol 5.00 ml Tween20 0.25ml and DH2O 4.75ml the solation 10ml. below given in the table 2.

Table 2. Essential oils different concentration

				5% Tween 20 (Fixed)						
%	**Essential oil**		**Ethanol**	**Tween 5%**		**DH_2O**		**Grand Total**		
4%	0.20	Ml	4.80 Ml	0.25	Ml	4.75	ml	10	Ml	
8%	0.40	Ml	4.60 Ml	0.25	Ml	4.75	ml	10	Ml	
12%	0.60	Ml	4.40 Ml	0.25	Ml	4.75	ml	10	Ml	
16%	0.80	Ml	4.20 Ml	0.25	Ml	4.75	ml	10	Ml	
0%	0.00	Ml	5.00 Ml	0.25	Ml	4.75	ml	10	Ml	

***In Vitro* Antifungal Effects of the Essential Oils:** Application of different essential oil of different concentration against Aspergillus Niger the essential oils use in Potato dextrose agar (PDA) media in pteria plates 18ml media 1ml essential oils mix 45°C then media are solidified then 5mm disc of *Penecillium sp.* mycelium placed on the treated PDA medium and pouring with parafilm and the plate incubate at 25°C mycelial growth determined each day to compare control petri plate. (Aminifard and Mohammadi, 2013a).

Statistical Analysis: The date was applied along with replication. For the statical tests such as Analysis of variance and LSD, the data was recorded and analyzed (Steel *et al*., 1997).

RESULT AND DISCUSION

Morphological identification of *Penecillium sp.:* The characteristics of, *Penecillium* sp. , like colony appearance and sporulation pattern were examined form cultures grown on media potato dextrose agar (PDA), white yellow color media later coming black conidia, colonies color in visible on isolated plates at 28°C for 6 days.

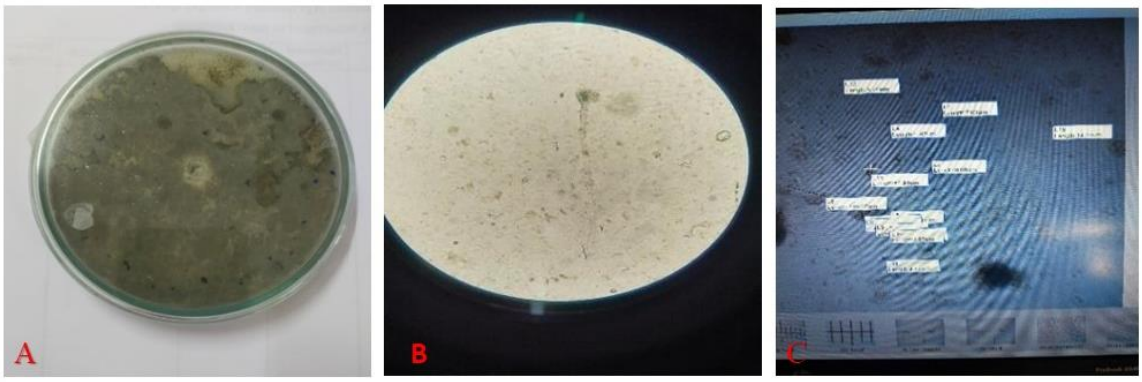

Figure. 1. A) Culture Plate of *Penicillium sp.* B) Microscopic Picture C) Microscopic Picture

Microscopic study of *Penicillium sp.:* As shown in Figure. 1. *Penicillium sp.* Conidiophores erect, unbranched, septate, at the apex with a vertical of erect primary branches, each with a verticil of secondary (metulate) and sometimes tertiary branch lets or with a vertical of conidia-bearing cells (phialides) borne directly on the slightly inflated apex of the conidiophores. Conidiophores about 300µ or 400 × 3.5-4µ, simple or rarely branched, with walls smooth or rough, penicilli long, asymmetrically arranged and 40-50µ long, branches about 1626 × 3-4µ, with walls occasionally roughened, in groups of two or rarely three; metulate about12-15 × 2.5-3.5µ in groups of three to five phialides about 9-10 × 2-2.5µ, in verticilis of five to ten. Conidia borne in chains which typically form a brush-like head, not enclosed in slime well differentiated foot cells not present. Conidia globose, ovate or elliptical, smooth or rough. Conidia 2.5-3.5 ×2.5-3µ smooth, variously ovate to subglobose, long, adhering in masses (Murmu, *et al*., 2020).

Pathogenicity Test: The bags were observed after 15 days of incubation at room temperature in the dark place. The results were qualitatively expressed by assessing the visual degree of growth and colonization of *Penecillium sp.* The following symbols indicate degrees of growth of the mold disease in the bags: (+): Poor growth, less than 20% of substrate colonization; (+ +): Intermediate growth, 20-50% of substrate colonization; (+++): abundant growth more than 50% of substrate colonization; (-): non-growth, same pathogen was observed with same symptoms as per results.

In vitro* evaluation of Cinnamon oil on mycelial growth *Penicillium sp: The data was collected after every two days of interval. All the data in table 3 was compared with control (C) having highest growth rates 29.78 mm due to non-application of the essential oils and 0% reduction of the colony growth. For the treatment of Cinnamon oil at 4% concentration, the highest total growth rate of the pathogen was 26.98 mm in petri plate and the least reduction in the colony growth percent was 9.40. Second concentration was 8% that exhibited 26.25 mm of growth and the reduction in the colony growth percent was 11.85. Third concentration with 12% indicated total colony growth of 20.67 mm and the reduction in the colony growth percent was 30.59. The least total growth of pathogen colony recorded at 16% concentration dosage was 20.60 mm and the highest decrease in the colony growth percent was 30.83.

The antifungal ability of cinnamon oil on *P. expansum* showed a dose-dependent manner. It did not affect the spore germination of *P. expansum* with a concentration of 0.05 mg L−1. When the concentration reached 0.25 mg L−1, cinnamon oil showed a stable inhibitory effect. After 12 h of treatment, the spore germination rate was below 20%, while it was over 75% in control. With concentration increasing, the inhibitory effect was more positive. With the extension of culturing, mycelial biomass production of *P. expansum* was significantly lower than that of control in PDB supplemented with 0.25 mg L−1 cinnamon oil, and the mycelial expansion and sporulation were significantly inhibited on PDA under cinnamon oil stress. For inoculation tests in vivo, decay symptoms were found in all inoculated apples, while lesion diameters in treated apples were significantly smaller than those in the control groupe. Therefore, 0.25 mg L−1 was considered as a minimum effective concentration of cinnamon oil on *P. expansum* and used in the subsequent experiments. (Lai, T. *et al*., 2021). The results show significant difference in the linear colony growth of *Penicillium*

sp. in which P value was less than 0.05 with application of Cinnamon oil concentrations.

Table. 3 *In vitro* efficacy of Cinnamon on colony growth of *Penicillium sp.*

Treatment		**Dose**	**Linerar Colony Growth (cm)**			**Total Growth**	**Reduction in Colony Growth (RCG)**	**Reduction Percentage**
			2nd Day	4th Day	6th Day		RCG=C-TCG	RCG(100)/C
Cinnamon		4%	8.83	22.15	26.98	26.98	2.80	9.40
		8%	7.32	18.05	26.25	26.25	3.53	11.85
		12%	7.22	17.63	20.67	20.67	9.11	30.59
		16%	6.88	17.03	20.60	20.60	9.18	30.83
Control (C)		0%	14.72	23.83	29.78	29.78	0.00	0.00

In vitro* evaluation of Coconut oil on mycelial growth *Penicillium sp: The data was collected after two days of interval. All the data in table 4 was compared with control (C) having highest growth rates 28.58 mm due to non-application of the essential oils and 0% reduction of the colony growth. For the treatment of Coconut oil at 4% concentration, the highest total growth rate of the pathogen was 22.88 mm in petri plate and the least reduction in the colony growth percent was 19.94. Second concentration was 8% that exhibited 15.02 mm of growth and the reduction in the colony growth percent was 47.46. Third concentration with 12% indicated total colony growth of 13.98 mm and the reduction in the colony growth percent was 51.08. The least total growth of pathogen colony recorded at 16% concentration dosage was 11.07 mm and the highest decrease in the colony growth percent was 61.28.

The inhibitory activity of different samples against *P. expansum* is shown in the growth the diameter of the *P. expansum* zone gradually decreased as the sample concentration increased, which indicated that (olive, coconut, essential oils) TAL and TAS essential oils and AITC also had varying degrees of inhibition on *P. expansum*. In addition, the analysis of the data showed when the concentration of TAS essential oil was 0.75 mg/mL, the inhibition rate on *P. expansum* reached 100%, while the inhibition rate of TAL essential oil on *P. expansum* was 98.60% ± 2.32%. When the concentration of TAL essential oil was 1.00 mg/mL, it also completely inhibited the growth of *P. expansum*, which indicates that it was more sensitive to TAS essential oil. AITC exhibited a strong inhibitory effect, with the inhibitory activity on *P. expansum* as high as 96.48% ± 2.45% with AITC at a concentration of 100 μg/mL. At a concentration of 125 μg/mL, the growth of *P. expansum* was completely inhibited. (Zhao, R., *et al*., 2022).

The results show significant difference in the linear colony growth of *Penicillium sp.* in which P value was less than 0.05 with application of Coconut oil concentrations.

Table 4 *In vitro* efficacy of Coconut on colony growth of *Penicillium sp*.

Treatment	**Dose**	**Linerar Colony Growth (cm)**			**Total Growth**	**Reduction in Colony Growth (RCG)**	**Reduction Percentage**
		2nd Day	4th Day	6th Day		RCG=C-TCG	RCG(100)/C
	4%	9.88	15.05	22.88	22.88	5.70	19.94
Coconut	8%	6.92	11.78	15.02	15.02	13.57	47.46
	12%	6.15	10.53	13.98	13.98	14.60	51.08
	16%	4.63	7.88	11.07	11.07	17.52	61.28
Control (C)	0%	12.10	18.95	28.58	28.58	0.00	0.00

In vitro* evaluation of Neem oil on mycelial growth *Penicillium sp: The data was collected after two days of interval. All the data in table 5 was compared with control (C) having highest growth rates 27.48 mm due to non-application of the essential oils and 0% reduction of the colony growth. For the treatment of Neem oil at 4% concentration, the highest total growth rate of the pathogen was 18.97 mm in petri plate and the least reduction in the colony growth percent was 30.99. Second concentration was 8% that exhibited 14.48 mm of growth and the reduction in the colony growth percent was 47.30. Third concentration with 12% indicated total colony growth of 13.82 mm and the reduction in the colony growth percent was 49.73. The least total growth of pathogen colony recorded at 16% concentration dosage was 6.98 mm and the highest decrease in the colony growth percent was 74.59.

Effect of different botanical extracts on the linear colony growth of *Penicillium expansum*: The results that minimum linear colony growth of *Penicillium expansum* was observed Neem 45.00, 38.00 and 33.66 mm, as Ginger at the doses of 5, 10 and 15%, respectively. The maximum linear colony growth 90mm was observed under control. The minimum linear colony growth of *Penicillium expansum* was

observed at 15% for Neem, respectively. Statistical analysis of the data revealed that there was a significant difference among the botanical extracts at a different level of concentration for the linear colony growth of fungus (Reki, M. A.*et al*., 2020).

The results show significant difference in the linear colony growth of *Penicillium sp.* in which P value was less than 0.05 with application of Neem oil concentrations.

Table 5 *In vitro* efficacy of Neem on colony growth of *Penicillium sp.*

Treatment	Dose	Linerar Colony Growth (cm)			Total Growth	Reduction in Colony Growth (RCG)	Reduction Percentage
		2nd Day	4th Day	6th Day		RCG=C-TCG	RCG(100)/C
	4%	8.58	15.08	18.97	18.97	8.52	30.99
Neem	8%	6.18	11.62	14.48	14.48	13.00	47.30
	12%	5.55	10.95	13.82	13.82	13.67	49.73
	16%	3.47	5.12	6.98	6.98	20.50	74.59
Control (C)	0%	9.88	21.38	27.48	27.48	0.00	0.00

In vitro* evaluation of Rose oil on mycelial growth *Penicillium sp: The data was collected after two days of interval. All the data in table 6 was compared with control (C) having highest growth rates 37.62 mm due to non-application of the essential oils and 0% reduction of the colony growth. For the treatment of Rose oil at 4% concentration, the highest total growth rate of the pathogen was 25.38 mm in petri plate and the least reduction in the colony growth percent was 32.54. Second concentration was 8% that exhibit 24.42 mm of growth and the reduction in the colony growth percent was 35.09. Third concentration with 12% indicated total colony growth of 19.38 mm and the reduction in the colony growth percent was 48.48. The least total growth of pathogen colony recorded at 16% concentration dosage was 21.08 mm and the highest decrease in the colony growth percent was 43.96.

Antifungal activity of the tested rose oil and petal extracts, three micro-organisms were tested; two molds (*Penicillium. notatum and A. niger*) and a yeast (*C. albicans*). The inhibition zones ranged between 10.5 to 17.5 mm. inhibition zones of C. albicans ranged between 10.5 and 14mm, while those of the *P. notatum* and *A. niger* ranged between, 12 to 17.5 and 11 to 17 respectively. *P. notatum* was the most sensitive and C. albicans was the least sensitive fungus Anti-fungal activity of the tested rose oil and petal extracts, microorganism was tested; *Penicillium. notatum*. The inhibition zones ranged between 10.5 to 17.5 mm. inhibition zones of the *Penicillium.*. notatum 12 to 17.5 respectively. *Penicillium.*. notatum was the most sensitive fungus (Shohayeb, M. *et al*., 2014). The results show significant difference in the linear colony growth of *Penicillium sp.* in which P value was less than 0.05 with application of Rose oil concentrations.

Table 6 *In vitro* efficacy of Rose on colony growth of *Penicillium sp.*

Treatment	Dose	Linerar Colony Growth (cm)			Total Growth	Reduction in Colony Growth (RCG)	Reduction Percentage
		2nd Day	4th Day	6th Day		RCG=C-TCG	RCG(100)/C
	4%	9.32	19.07	25.38	25.38	12.24	32.54
Rose	8%	8.60	15.85	24.42	24.42	13.20	35.09
	12%	6.98	14.45	19.38	19.38	18.24	48.48
	16%	6.40	16.02	21.08	21.08	16.54	43.96
Control (C)	0%	10.35	19.13	37.62	37.62	0.00	0.00

CONCLUSION

It was concluded that *Penecillium sp.* effects the substrate and ultimate result of which is less yield of mushrooms and low quality mushrooms. The current study is carried out to determine *in vitro* evaluation of *Penecillium sp* contaminant in Spawn culture of mushroom and management through essential oils and results showed that of mycellum inhibition of *Penecillium sp*. was highly effected by Neem oil followed by coconut oil, Rose oil and Cinnomon oil.

REFERENCES

Aminifard, M., & Mohammadi, S. (2013a). Efficacy of plant essential oils to control postharvest decay of sweet cherry (*Prunus avium* L.) fruit. J. Hortic. Sci. Biotechnol. 88 (1):79–84.

Colavolpe, M. B., Mejía S.J., Alberto, E. (2014). Efficiency of treatments for controlling *Trichoderma* spp during spawning in cultivation of lignicolous mushrooms. *Brazilian journal of Microbiology*, **45,** 1263-1270.

Ghimire, A., Pandey, K.R., Joshi, Y.R., & Subedi, S. (2021). Major fungal contaminants of mushrooms and their management. *International Journal of Applied Sciences and Biotechnology*, **9**(2)., 80-93.

Gupta, S., Summuna, B., Gupta, M., & Annepu. S. (2018). Edible Mushrooms: Cultivation, Bioactive Molecules, and Health Benefits. Bioactive Molecules in Food, 1-33.

Hamid, O., Robert, C., Daud, A., Hodi, F. S., Hwu, W. J., Kefford, R., & Ribas, (2013). Safety and tumor responses with lambrolizumab (anti–PD-1) in melanoma. *New England Journal of Medicine*, **369**(2), 134-144.

Joseph, M. (2021). Types of edible mushroom. Retrieved from www. Nutrition advance. com: http://wwww.nutritionadvance.com.

Lai, T., Sun, Y., Liu, Y., Li, R., Chen, Y., & Zhou, T. (2021). Cinnamon oil inhibits *Penicillium expansum* growth by disturbing the carbohydrate metabolic process. *Journal of Fungi*, **7(**2), 123.

Murmu, R., Maurya, A.K. & John,Y. (2020). Mycoflora of certain casing materials used in the production of whight button mushroom (*Agricus bisporus*) (*lanige imbach*). Internation Journal of chemical studies, **8**(2), 2863-2868.

Rathore, H., S. Prasad, M. Kapri, A. Tiwari., & S. Sharma. (2019). Medicinal importance of mushroom mycelium: mechanisms and applications. J. Funct. Foods 5(6), 182–193.

Reki, A., & Okicic, N. (2020). On kuratowski measure of Noncompactness in r 2 with the river metric. *Balkan Journal of Applied Mathematics and Informatics*, **3**(2), 59-68.

Rosario, C. J., Rani, M., & Joseph, S. (2021). Biological evaluation and methods of cultivation practices of selected oyster mushroom using different substrates in Nirmala College Campus, Coimbatore, Tamilnadu **4(**2), 160-167.

Shohayeb, M., Abdel-Hameed, E. S. S., Bazaid, S. A., & Maghrabi, I. (2014). Antibacterial and antifungal activity of Rosa damascena MILL. essential oil, different extracts of rose petals. *Global Journal of Pharmacology*, **8**(1), 1-7.

Steel, R. G., Torrie, J.H., & Dickey, D.A. (1997). Principals of Statistics, A Biological Approach. 3rd Ed. McGraw Hill, Inc. Book Co. N.Y. (U.S.A.), 352-358.

Zhao, R., Ben, A., Wei, M., Ruan, M., Gu, H., & Yang, L. (2022). Essential oil obtained from *Thlaspi arvense* L. leaves and seeds using microwave-assisted hydrodistillation and extraction in situ by vegetable oil and its antifungal activity against *Penicillium expansum*. *LWT*, **16**(5), 113718.