Research Article

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Antifungal property of essential oil extracted from *Zanthoxylum armatum (Timur)*

Abstract

The aim of this research work was to test the effect of essential oil of *Zanthoxylum armatum (timur)* originated from Salyan (Nepal) against five isolated fungi i.e. *Aspergillus sp., Alternaria sp., Penicillium sp., Cladosporium sp.* and *Helminthosporium sp.* The essential oil yield of sample from Salyan was 2.29%. Different concentrations i.e. 5µl, 10µl, 15µl and 20µl of essential oil were used to determine zones of inhibition. The zone of inhibition was highest against *Cladosporium sp.* at all concentrations, while it was lower against *Penicillium and Helminthosporium sp.* MIC values of essential oil of *Zanthoxylum armatum* were found to be 0.16mg/ml, 0.14mg/ml, 0.4mg/ml, 0.1mg/ml and 0.4mg/ml against *Aspergillus sp, Alternaria sp, Penicillium sp, Cladosporium sp* and *Helminthoaporium sp* respectively.

Keywords: *Zanthoxylum armatum,* minimum inhibitory concentration, zone of inhibition, anti-fungal property

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Nabina Prajapati,[1] Pravin Ojha,[2] Tika Bahadur Karki[3]

[1]National College of Food Science and Technology, Nepal
[2]Food Research Division, Nepal Agricultural Research Council, Nepal
[3]Department of Biotechnology, Kathmandu University, Nepal

Correspondence: Pravin Ojha, Food Research Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal, Tel +9779851023022, Email projha84@gmail.com

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Introduction

Timur (*Zanthoxylum armatum)* is a major indigenous spice of Nepal. The ripe fruit follicles are usually reddish in color and 4 to 5mm in diameter. The dried fruit also contain an aroma that is present in brown fruit wall (pericarp-shell). It may be able to develop numbing or anesthetic feeling on the tongue. Seeds are solitary, globose, shining and have bitter taste.[1] *Timur* is not only used as flavouring in cooking but also its seed oil and crushed seeds are added to cereal seeds and legumes to protect them against damages caused by stored grain pests.[2] *Timur* is the main non-timber forest product of Nepal. This spice is mostly cultivated in the mid-western region of Nepal. Among 850-1100tonnes of *timur* harvested in Nepal, 400 tonnes of *timur* are collected alone in Salyan district.[3]

Loss of nutrient result in loss of quality, quantity and monetary value is due to toxigenic strains of mold during post-harvest storage and processing.[4] Ten percentage of agricultural food commodities in the world are severely spoiled by molds to the extent that they are no fit for consumption.[5] Molds like *Aspergillus* sp., *Penicillium, Cladosporium, Alternaria, Helmenthosporium,* etc produce mycotoxins among which aflatoxin was found to have carcinogenic risks. Hence, aflatoxin contamination represents a serious threat to food contamination in both developing and developed countries.[6] The spoilage of foods as a result for contamination with such molds can be prevented by the use of several spices.[7] The essential oil of fruits of *Z. armatum* exhibits good antibacterial, antifungal and anthelmintic activities.[8] The largest zone of inhibition was obtained against *Bacillus subtilis* (23 mm) and minimum bactericidal concentration (MBC) value of 2.5mg/L was obtained.[9] Essential oil of it is assessed for their fungitoxicity against *Alternaria brassicicola*.[10]

However, data are lacking regarding effectiveness of essential oil (*timur*) against food spoilage fungi. Thus, the aim of this study was to assess the antifungal activity of essential oil extracted from *timur* against several fungi. Essential oil production of *timur* and its utilization can add value to this indigenous spice as well.

In the present study, essential oil of *timur* was tested against five molds that can contaminate foods. The purpose of this was to create directly comparable, quantitative, antifungal data and to generate data for the essential oil of *timur*. This study suggests that the essential oil of *timur* has some antifungal property.

Materials and methods

Collection of *timur* and extraction of essential oil

The *Timur* (dry fruit) was collected from the local market (Asan) of Kathmandu valley, which was from the districts of mid-western regions of Nepal (Salyan) shown in Figure 1. The *timur* were grinded by the use of grinder to the coarse size of 75 mesh size (IS standard) and stored in an airtight containers under refrigerated condition (4°C) until use. The ground sample of t*imur* (50g) was hydro distilled in a Clevenger apparatus for 5-6hours.[11] The volatile oils were stored in sealed vials at 4°C until analysis as shown in Figure 2. The yield of the oils was calculated based on dried weight of powdered samples of *Timur*.

Figure 1 Dry fruits of *timur.*

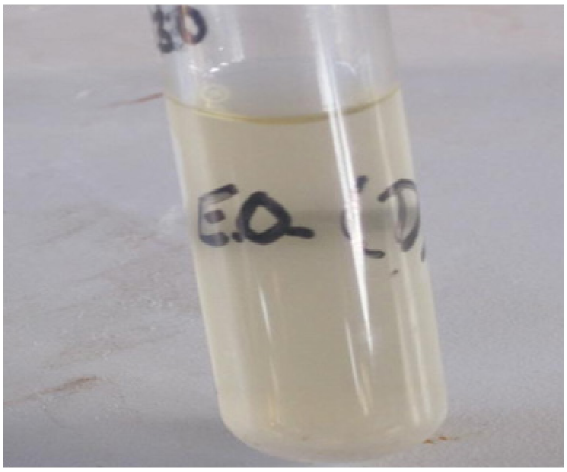

Figure 2 Essential oil of *timur*.

Isolation and identification of the fungi

The fungi were isolated from soil and the spoiled meat, vegetables and fruits in media potato dextrose agar by spread plate technique at temperature 25^{0}C for 5 days. Then, the color of different shapes and colonies were further point inoculated on the plates of potato dextrose agar and again incubated at 25˚C for 5days.[12] The isolated fungi were then microscopically examined and the fungi were identified in accordance with Benson.[13]

Determination of zones of inhibition

A sterile wire loop was used to place the test fungi into a test tube with sterile water over an open flame to obtain fungal suspension in the sterile water.[14] The prepared inoculums of fungi were swabbed all over the surface of the PDA plate using sterile cotton swab. Two wells of 6mm diameter were bored in the medium with the help of sterile cork-borer having 6mm diameter and were labelled properly. The essential oil of *timur* sample was filled in one of the wells with the help of micropipette. Different doses of the essential oil were used i.e. 5µL, 10µL, 15µL and 20µL for the test against each fungi. The next well was filled with same volume of the sterile water as the negative control. Plates were left for some time until the essential oil diffused in the medium with the lid closed and then, incubated at 25°C for 5days. The zone of inhibition was measured by using scale ruler and means were recorded after incubation.[9]

Determination of minimum inhibitory concentration (MIC)

0.01 to 20mg/mL of the essential oil was prepared by using 100% dimethyl sulfoxide (DMSO).The prepared essential oil ranging from 0.01 to 20mg/mL were added to the well of each petri dish along with DMSO, as control. Further, it was incubated at 25°C for 5days and then, inhibition of the growth organism was observed and MIC was determined.[15]

Statistical analysis: All the data obtained in this experiment were analyzed by statistical program Genstat release 7.22, VSN International Ltd. Sample means were compared by LSD method at 95% level of significance.

Results and discussion

Yield of essential oil

The essential oil of the samples of *timur* extracted by the hydro-distillation method was light yellow in color. Essential oil yield for the *timur* of Salyan was found to be 2.29±0.05%. The findings were greater than that of Tiwary et al.,[2] and Prakash et al.,[16] but less than that of Verma and Khosa.[17] The difference might be due to differences in *timur* variety.

Isolation and identification of fungi

Different spoiled food materials and soil were used for the isolation of fungi. Five different strains of fungi namely Aspergillus sp., Penicillium sp., Alternaria sp., Cladosporium Sp. and Helminthosporium sp. as shown in Figures 3–7 respectively were isolated from these and their morphological characteristics were illustrated in Table 1.

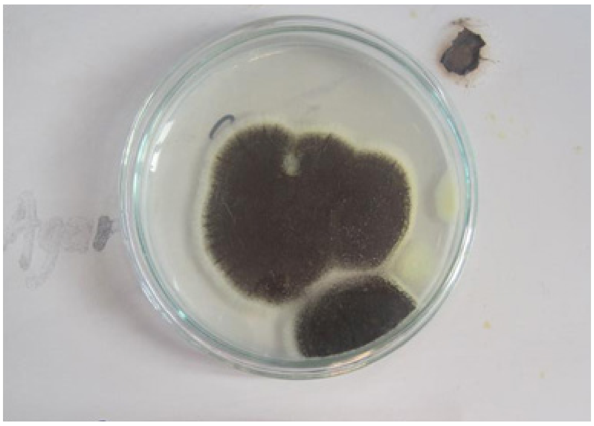

Figure 3 *Aspergilus sp. on PDA plate.*

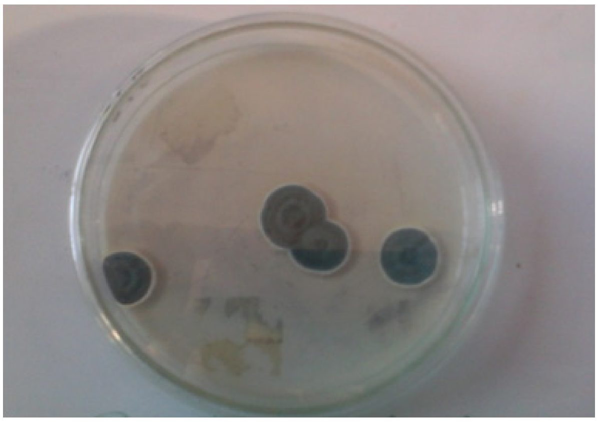

Figure 4 *Penicillium* sp. on PDA plate.

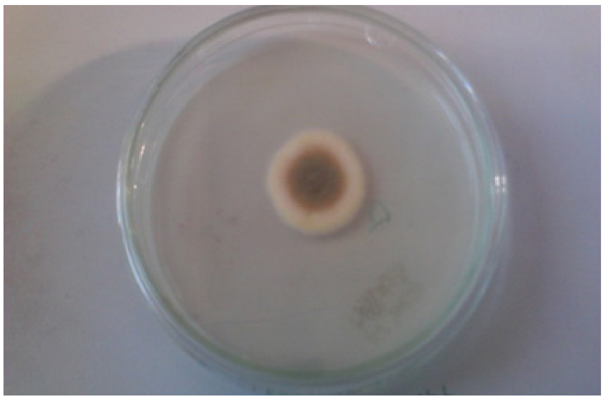

Figure 5 *Alternaria* sp. on PDA plate.

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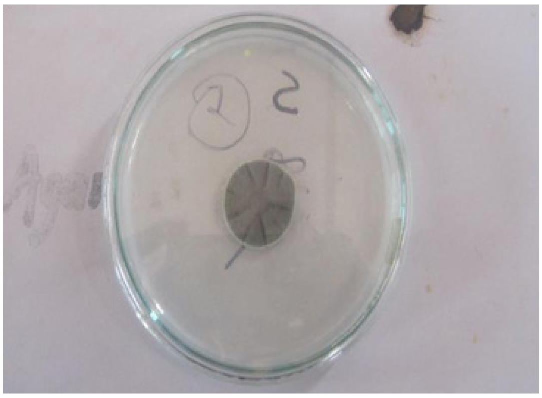

Figure 6 *Cladosporium* sp. on PDA plate.

Figure 7 *Helminthosporium* sp. on PDA plate.

Zones of inhibition

Essential oil extracted from different samples of *Zanthoxylum armatum* were tested separately against all five microorganisms. In every test it was clear that the essential oil showed highest inhibition against *Cladosporium sp* at all the concentrations and comparatively lower against *penicillium* sp and *Helminthosporium* sp as shown in Figure 8. The result show that with increase in concentration, the zone of inhibition for all species increases.

Nanasombat and Wimuttigosol[18] found that essential oil extracted from fruit of *Zanthoxylum limonella* (Rutaceae) at concentration of 10µl/disc show zone of inhibition of 42.3, 34.0, 33.3, 25.8 and 23.5mm against *Rhodotorula glutinis*, *Schizosaccharomyces pombe*, *Hanseniaspora uvarum*, *Aspergillus ochraceus* and *Fusarium moniliforme* respectively. The result was different from that for *Aspergillus*, which may be due to difference in variety.

According to Prakash et al.,[16] the essential oil of another variety of *Zanthoxylum* i.e. *Zanthoxylum alatum* exhibit a broad range of antifungal activity against mold like *Aspergillus flavus*, *niger, terreus*, *candidus*, *sydowi*, *fumigates*, *Alternaria alternate*, *Cladosporium cladosporioides*, *Curvular ialunata*, *Fusarium nivale*, *Penicillium italicum* and *Trichoderma virdie*. By the chromatographic method against *Alternaria alternate*, *Zanthoxylum alatum* showed the zone of inhibition (RF of 0.51) of 18mm diameter.[19]

Tiwary et al.,[2] found linalool (57%) and limonene (19.8%) as major component of essential oil of *Zanthoxylum armatum.* The antifungal activity of essential oil is generally due to linalool content.[20] Limonene, however show effective antimicrobial activity but possess moderate antimicrobial activity.[21]

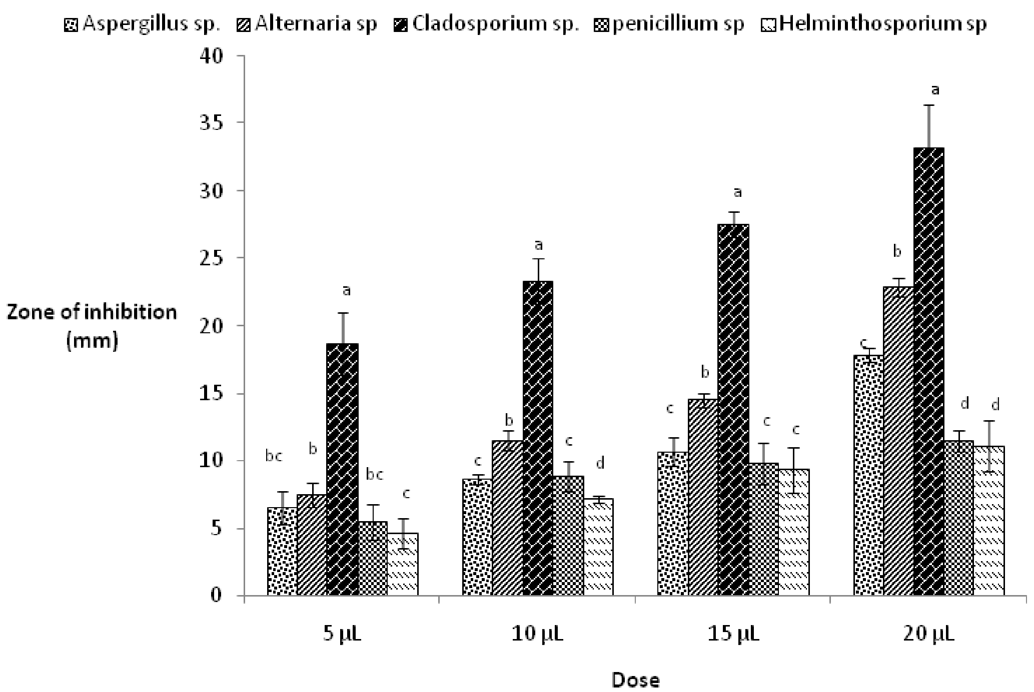

Figure 8 Effect of concentration of essential oil on isolated fungi species.

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Minimum inhibitory concentration (MIC)

The MIC value of essential oil of *Zanthoxylum armatum* for *Aspergillus* sp was0.16 mg/mL. For *Alternaria* sp., it was 0.14mg/mL; while MIC value for *Penicillium* sp. and *Helminthosporium* sp. was same i.e. 0.4mg/mL. Similarly, MIC value for *Cladosporium* sp. was found to be 0.1 mg/mL. The MIC value for the *Cladosporium* sp. was the lowest and for the *Penicillium* sp. and *Helminthosporium* sp., it was highest among the test fungi as shown in Table 2.

Table 1 Morphological characteristics of isolated fungi

Fungi	Morphological characteristics
Aspergillus sp.	Grayish black in color; reverse side white to yellow; cottony in texture; hyphae branched like fan or tree, rough irregular conidia surface
Penicillium sp.	Bluish green in color; reverse side yellow in color;Velvety in texture; brush arrangement of philaspores.
Alternaria sp.	Gray green in color at center; cottony in texture; chains of microconidia
Cladosporium sp.	Olive-gray in color; reverse side gray to black in color; Dark blast conidia different in shape.
Helminthosporium sp.	Black surface with grayish periphery; macroconidia shown.

Table 2 MIC value required by the *Zanthoxylum* oil to inhibit the test fungi

Test fungi	Minimum inhibitory concentration(mg/ml)
Aspergillus sp.	0.16
Alternaria sp.	0.14
Penicillium sp.	0.4
Cladosporium sp.	0.1
Helminthosporium sp.	0.4

Nanasombat and Wimuttigosol[18] reported MIC value 1mg/mL for *Aspergillus ochraceus* and *Fusarium moniliforme* against essential oil extracted from fruit of *Zanthoxylum limonella* (Rutaceae). Yang and Chen[22] reported MIC values of 25 and 6.25µg/ml for *Cladosporium cucumerinum* and *Pyriculeri aoryzae* against dictamnine extracted from steam of *Zanthoxylum nitidum*. EOs rich in oxygenated monoterpenes have been shown to possess antifungal activities[19] and the essential oil of *Zanthoxylum armatum* is rich in oxygenated monoterpenes (75%).[2] Many researchers have investigated the antifungal and antibacterial activities of individual chemical constituents of the EOs such as β-caryophyllene, caryophyllene oxide, and linalool etc;[23,24] and *Zanthoxylum* contains high amount of linalool.[16]

Conclusion

The essential oil of *Zanthoxylum armatum* shows antifungal property against various fungi like *Aspergillus* sp., *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp. and *Helminthosporium* sp. Among the test fungi, it was most effective against *Cladosporium* sp. and comparatively less effective against *Penicillium* and *Helminthosporium* sp. As *Timur* has antifungal property, it can be used as preservative in food commodities to prevent fungal growth. Further, synergistic effect of *timur* oil with other spices oil can be studied.

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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