Comparative Efficacy of Some Edible Salt Varieties against *Aspergillus niger*

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Abstract: ***The study envisages the screening of some edible salt varieties like table salt, Rock salt and Himalayan salt for their efficacy against food spoilage fungal pathogen Aspergillus niger. Different concentrations of the salt varieties were prepared and using disc diffusion methods were screened for their efficacy. It was found that almost all the salt concentration had good antifungal activity. The mycelial growth of the fungus was significantly suppressed in rock and Himalayan salt at all concentrations and comparatively the process was lower in table salt. The study shows that the Himalayan salt was the best antifungal agent compared to rock salt and table salt. Further studies with more concentration will give an idea on its threshold capacity.***

Keywords: Antifungal study, rock salt, Himalayan salt, table salt, *Aspergillus niger*

1. Introduction

Food loss, causes considerable environmental and economic damage wherein fresh produce accounted for nearly 20% of this loss (Alimelli et al., 2007).Generally, about 30 % fruits and vegetables are rendered unfit for consumption due to spoilage after harvesting. India annually produces fruits and vegetables of the value of about Rs. 7000 crores and wastage may be of the order of Rs. 2100 crores. This is a huge loss of valuable food even when the minimum food requirement of the population is not met. The issue of food losses is of high importance as they have an impact on food security for poor people, on food quality and safety, on economic development and on the environment. Fungal spoilage of dairy foods is manifested by the presence of a wide variety of metabolic by-products, causing off-odours and flavours, in addition to visible changes in colour or texture (Gram et al 2002). *Aspergillus* and related molds generally grow faster and are more resistant to high temperatures and low water activity. That's why they are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices (Cairns etal., 2015). As a consequence of limited antifungal therapeutic options and timely diagnostics, mortality rates can be extremely high, caused by invasive aspergillosis or other systemic mycoses (Meyer etal., 2016). Several types of modified atmosphere packaging (MAP) have been developed to retard growth of pathogenic and spoilage organisms (Guynot et al., 2003; Dalgaard et al., 2006; Ercolini et al., 2006). However, microbes are endlessly innovative and eventually seem to circumvent the barriers we set up against them. Therefore, further strategies and multiple hurdles are utilized to extend the shelf life, but these procedures must be assessed for compatibility with different foods so that there are no significant organoleptic changes in the foods caused by the treatment of preservatives. From time immemorial salt is an important part of our food and they are essential for functioning of our brain and nerves as they support electrical impulses in the body. Salt is commonly used to flavour the food, and as food preservatives. Sea salt or Rock salt (*Sendha namak)* in its purest form is made of Sodium Chloride, but unrefined and depending on its source it contains various trace minerals like potassium, zinc, manganese, calcium and iron. It can be used as a healthy substitute of common salt as it is rich in minerals and does not pose health problems like high blood pressure, puffiness in the body or eyes. Rock salt Lamps serve as natural air ionizers that can remove harmful ions from air we breathe (Sarker et.al., 2016). Himalayan salt is rock salt or halite mined at the Khewra Salt Mine in Khewra in Punjab region of Pakistan. It is similar to table salt plus mineral impurities and consists of 95-98% sodium chloride, 2-4% polyhalite (potassium, calcium, magnesium, sulphur, oxygen, hydrogen), 0.01% fluoride, 0.01% iodine and small amounts of numerous trace minerals wherein the impurities in some veins of salt give it a pink, reddish, or beef red colour (Sarker et.al., 2016). There are reliable reports showing around 25 nutrients and non-nutritive minerals in pink salt (Shah, 2009, Rahman et al 2015, Flavia Fayet - moore et. al., 2020). Table salt or iodised salt is the most common type of salt used in home kitchens as it dissolves easily. It is an industrial salt refined to about 99 percent sodium chloride and contains additives to prevent it from clumping together. New type of salt has recently appeared in the market and they claim to be less harmful to health than current commercial salt (Carapeto et.al., 2018). Sodium chloride is a known antifungal agent used routinely in the aquaculture industry (Stockwell et.al., 2012). Similarly other salts like Ammonium Phosphate, Potassium carbonate, Potassium bicarbonate, Sodium carbonate and Sodium carbonate inhibits the growth rate of *Fusarium solani* (Ghadiri et.al., 2013). On the other hand, suppressive effect of sodium and calcium salts applied individually (or) combined with the yeast *Saccharomyces cerevisiae* was reported against *Alternaria solani* (El-Mougy et.al., 2009). The efficacy of salts such as Potassium phosphate dibasic sodium chloride, sodium sulphate and trisodium phosphate as possible alternatives to synthetic fungicides for the control of *fusarium oxysporum*, were also reported (Turkkan, 2013). Similarly inorganic salts viz., Ammonium molybdate, Ammonium oxalate, Cupric sulphate, EDTA, Ferric Chloride, Ferrous sulphate, Potassium meta bi sulphate and Zinc sulphate were tested against *Fusarium solani, Fusarium oxysporum, Rhizoctonia solani* and *Phytophthora infestans* (Rani et.al., 2016). A laboratory experiment was conducted to study salts types and effect of their concentrations on the growth of *Aspergillus niger* and *Penicillium oxalicum* (Waheed et al., 2019**)**.Likewise, a study was undertaken to determine and compare the sodium and potassium contents in selected salts (table salt, coarse salt, French sea salt, Himalayan pink salt and bamboo salt) (Tan et al., 2016).Based on the information cited above this study tries to screen some of the common edible salt varieties for their potential efficacy against a common food spoilage fungus *Aspergillus niger*.

2. Methodology

Aspergillus niger isolate was used in this study. The cultures were locally isolated from spoilt bread mold, identified by standard procedure and confirmed with the culture collection maintained by local expertise. The fungal strains were maintained on Potato Dextrose Agar (PDA) slants and stored at 4ºC, which served as stock cultures for future use. The disc diffusion assay method of Iennette (1985) as described by Rabe & Van Staden (1997) was used with some modifications to determine the rate of inhibition in growth of the fungus in different salt concentrations. The diluted fungal cultures were spread over PDA plates using sterile L-rods. About 0.2 ml of each of the extract was applied in Whatman's No:1 filter paper discs (5 mm diameter) and allowed to dry before being placed on the top layer of the PDA plate containing the Aspergillus colony. The plates were incubated at 27ºC for 72 hours and the diameter of inhibition zones were recorded.

Three different edible salt varieties were used for this study i.e. Table salt, Rock salt and Pink (Himalayan) salt. The salt types were obtained from local shops, where it was easily available. They were sterilized in autoclave for 15 minutes at 121ºC at 15 lbs. and stored before use. The salt varieties were diluted to 5, 10, 15 % (w/v) using distilled water and stored aseptically before application as per standard procedures. The effect of the salts on the mycelial growth of the fungal species were assayed according to Mecteau *et al* (2002), with a slight modification. Discs dipped in distilled water was maintained as control.

The fungal mycelium was transferred from 7-day old cultures in the centre of the plate and the plates were sealed with parafilm and incubated at 24ºC. Mycelial growth was measured every day until the growth reached the edge of the petri plates. Each treatment was replicated 3 times and the experiment was repeated twice. The mycelial growth was determined in PDA amended with salts at concentrations of 5, 10, 15 % (w/v) for each salt variety respectively. The results were recorded by measuring the zone of growth inhibition around the disc measured from centre and was expressed in mm diameter.

3. Results and Discussion

This study evaluated the inhibitory activity of different salt varieties against *Aspergillus niger*, a common food spoilage fungus at different concentrations (Table 1; Fig. 1; Plate 1).

Table 1: Inhibition zone diameter for different salt concentration against *Aspergillus niger*.

Salt variety	Inhibition zone diameter (mm) / salt Concentration (%)				Average (mm)
	5	10	15	Control	
Table salt	6	6	7	0	6.3
Rock salt	7	9	11	0	9.0
Himalayan salt	9	12	15	0	11.6

The different salt varieties had some potential antifungal activities against the test pathogen. The table salt had the least inhibition potential among the different salts tested. It is reported that there was minimum inhibition at 5% concentration and increased only at the highest test concentration of 15%. The average inhibition was around 6.3 mm. it shows that the common table salt, since it is a refined product lacks natural antifungal characteristics. It is suspected that the supplementation of Iodine in the table salt contributes to the antifungal effects.

The rock salt on the other hand has a significant inhibition rate compared to table salt wherein it showed a significant inhibition zones in all the concentration studies with a peak at 15%. The significant increase may be due to its unrefined state. The average inhibition was around 9 mm. Comparatively it can be said that rock salt in spite of its crudeness and low user friendliness, is a capable antifungal agent. Nevertheless, it is used for most of the preservation processes.

Himalayan salt is known for its therapeutic characteristics. It has not failed here too as the test results show a significant inhibition zone in its lowest concentration and it was quite more than the averages shown by table salt or rock salt. The inhibition zone increases significantly for all the test salt concentrations and peaked at 15% with the maximum of 15mm. It shows that the pink salt or the Himalayan salt has the most antifungal capability compared to the other two test salts.

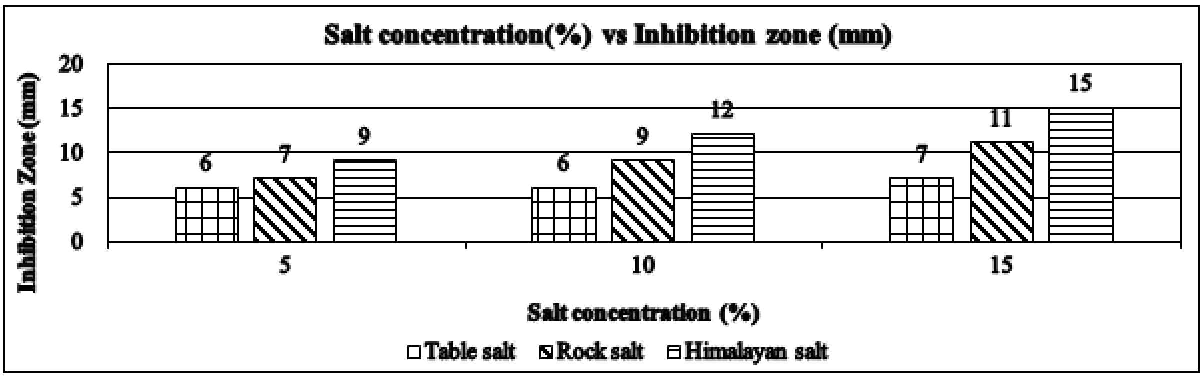

Figure 1: Inhibition zone diameter for different salt concentration against *Aspergillus niger.*

There had been numerous studies with many non-edible commercial salts against some pathogenic fungi, which had similar antifungal characteristics, some of which include sodium salts (Mecteau et al 2002). Comparatively the sodium salts used by them had significant antifungal potential. It can be said that this study to had similar antifungal characteristics with edible salt i.e. Sodium chloride. Similar results with Sodium chloride was reported by Waheed et al in 2019 who showed significant *Aspergillus* mycelial growth suppression on increase in salt concentration.

In another study Abdel-Kader et al (2012) too showed gradual increase in inhibition zones using calcium chloride, wherein it reduced the mycelial growth of *Fusarium solani*. However, Reid et al (2001) reported that sodium chloride was more effective than other chloride salts in controlling *Fusarium oxysporum*. Similarly, Amira et al., (2021) report the effect of some salts on sweet pepper, which has the mention of Sodium salt as good inhibitor of *Aspergillus niger* among other pathogens too. This report confirms the present study that NaCl is a natural antifungal agent.

But in contrast, in a study by Palmer et al (1997) and Turkkan (2013) had reported that sodium chloride did not have significant antifungal potential i.e. it did not reduce the mycelial growth of some fungal species, but stimulated its growth. This is consistent with Juniper and Abbot, (2006) study, which confirmed that salinity inhibits both of spore growth and mycelium formation. On the contrary Fungi is also known to be tolerant of salt concentration but to some extent. Similar were the results of (Madigan and Marrs, 1997) who found that the fungi use salts with concentrations of less than 10% in the current of cellular proteins with high levels of sodium chloride in the outer medium in order to achieve the required balance for salt adaptation.

To conclude the study, it is reported that the mycelial growth of the fungus was significantly suppressed in rock and Himalayan salt at all concentrations and comparatively the process was lower in table salt. The inhibition factor was significant in Rock salt and was highest in Himalayan salt. The study shows that the Himalayan salt was the best antifungal agent compared to rock salt and table salt. It can be attributed to the unrefined nature of the rock salt and Himalayan salt as it contained more minerals along with sodium, iron and zinc, so it can be concluded that Himalayan salt is beneficial to our health and is recommended for safe consumption.

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PLATE 1

Effect of different concentration of salt varieties on *Aspergillus niger*