



# Studying the Effect of *Foeniculum Vulgare* Mill and *Ziziphora Clinopodioides* Lam. Extracts on the Growth of *Aspergillus Flavus* Mold in Tomato Paste and Predicting the Data Obtained Using Artificial Neural Networks

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## ABSTRACT

The antifungal activity of *Foeniculum Vulgare* Mill and *Ziziphora clinopodioides* Lam. extracts against *Aspergillus flavus* in tomato paste containing different percentages of the extracts was tested. To this end, *Foeniculum Vulgare* Mill and *Ziziphora clinopodioides* Lam. extracts with different concentrations of 0.5, 1 and 2% were prepared and studied during different storage times (35 days). The effect of extracts of *Foeniculum Vulgare* Mill and *Ziziphora Clinopodioides* Lam with different concentrations was investigated alone in the environment (in vitro). By injecting 0.1 ml of mold in Sabouraud dextrose agar broth culture medium, then placing it in an incubator temperature of  $25^{\circ}\text{C} \pm 0.5$ , it was kept for 5 weeks (35 days), and one culture was done every week in order for the activity mold to be investigated in different concentrations of extracts. The results of antifungal activity of different levels of the extracts indicated that treatments 3 (containing 2% *Foeniculum Vulgare* Mill extract) and 4 (containing 3% *Foeniculum Vulgare* Mill extract) were resistant to the growth of *Aspergillus flavus* mold mycelium until the end of storage period. Generally, it can be concluded that using 2 or 3% *Foeniculum Vulgare* Mill extract as a natural preservative in tomato paste has a desirable antifungal activity. Artificial neural network was used to validate and evaluate the results of the experiments in predicting the data of *Aspergillus flavus* mold growth in tomato paste. In the present study, two hidden layers with 30 neurons were used. The network had two inputs including extract concentration and storage time, and the growth of *Aspergillus flavus* mold was considered as the target. Evaluation parameters such as correlation coefficient, mean squared error and maximum error showed very good results with values of 0.9993, 0.10934 and 0.13538. The lower the error and the closer the correlation coefficient to 1, the better the prediction is.

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## 1. Introduction

Plant extracts are known as a source of natural antioxidants and antimicrobials. Among the main sources of natural antioxidants are oregano, mint, thyme and cinnamon, which have been used as food preservatives for centuries and are considered medicinal plants. These antioxidants have positive effects such as protection against chronic diseases, cancer, diabetes, heart diseases and mutagenesis. The use of natural plant-based antioxidants in various sectors of the food industry has been proven as an effective factor in postponing chemical and oxidative changes and increasing the shelf life of products. The composition, structure and functional groups of essential oils and extracts play an important role in their antimicrobial and antioxidant activity, and usually the compounds that contain phenolic groups are more effective [1-4]. Kalantari et al.[5] evaluated the antifungal activity of cinnamon and wild marjoram essential oil on *Aspergillus flavus* in culture medium and tomato paste. The results showed that cinnamon and wild marjoram essential oil at concentrations of 200 and 500 ppm, respectively, are able to completely inhibit the growth of *Aspergillus flavus* in the culture medium. In contrast, 100 ppm of cinnamon and wild marjoram essential oils had 86.7 and 82% inhibition in tomato paste, respectively. Omid Bigi et al. [6] studied the activity of thyme, marze and clove essential oils on *Aspergillus flavus* in liquid medium and tomato paste. The results showed that all essential oils can prevent the growth of *Aspergillus flavus*, and thyme and marze oils had the strongest inhibition at 350 ppm and 500 ppm, respectively. Olaniran et al. [7] evaluated and studied the biological preservation effect of ginger and garlic powder on the properties of tomato paste. The results showed that the number of live cells and lactic acid bacteria in the control sample ranged from 3.42 to 13.45 and from 5.79 to 17.74 (log CFU/g), respectively, while the samples containing garlic and ginger had a number of Viable cell ranged from 3.34-87.4 to 3.39-4.86 (log CFU/g) during the storage period. According to the results of

this study, the combination of garlic and ginger (2.4%) prevents tomato paste from spoiling for 8 weeks at refrigerator temperature ( $4\pm 1^\circ\text{C}$ ). Tabatabai Yazdi et al. [8] in a study investigated the antimicrobial effect of extracts of dark mint plants (thyme, peppermint and kakuti) in preventing the growth of *Staphylococcus aureus* and *Geotrichum candidum* in industrial buttermilk samples. For this purpose, 3 concentration levels of each extract (0.15, 0.075 and 0 volume units) were prepared. After examining and comparing the results of the extract of natural inhibitory compounds in preventing the growth of *Staphylococcus aureus* and *Geotrichum candidum* in buttermilk samples, it was determined that the extract of dark mint plants had a greater effect on reducing the population of *Staphylococcus aureus*. Sidim et al. [9] Antimicrobial properties of whey protein isolate film containing 1-4% (weight-volume) of oregano, rosemary and garlic essential oils against *E.coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Lactobacillus plantarum*. Measure the contract. The inhibition halos were measured after the greenhouse period. The film containing oregano essential oils was more effective against bacteria at the 2% level than other films containing rose marigold extract and garlic. The use of Rose Marie essential oils along with whey protein isolate film did not show any antimicrobial activity, while the inhibitory effect of films containing garlic essential oils was observed only at 3 and 4% levels. Hossein et al. [10] Antifungal effect of Jedwa plant extract and phenolic compounds<sup>1</sup> have been investigated. The molds studied in their research are *Fusarium*<sup>2</sup>, *Trichoderma conyengi*<sup>3</sup>, *Penicillium*<sup>4</sup>*Ganoderma tropicum*<sup>5</sup>*Ganoderma lucidum*<sup>6</sup>*Aspergillus*<sup>7</sup> and *rhizopus*<sup>8</sup> have been. The most antimicrobial properties have been observed in the methanolic

- 
1. *Barringtonia racemosa*
  2. *Fusarium* sp
  3. *Trichoderma koningii*
  4. *Penicillium* sp
  5. *Ganoderma tropicum*
  6. *Ganoderma lucidum*
  7. *Aspergillus* sp
  8. *Rhizopus* sp

extract, which had the greatest inhibitory effect on the growth of the mentioned molds in the test. Methanolic extract from the leaves of Jedva plant has the most inhibitory effect on: *Fusarium* (53.45%), *Ganoderma lucidum* (34.57%), *Aspergillus* (32.27%) and *Trichoderma koningii* (20.99%) respectively. has shown itself. *Fusarium* mold has shown the most sensitivity to the aqueous extract of Jedva plant leaves. Also, the ethanolic extract of Jedva leaves had the most inhibitory effect on *rhizopus* mold. Aqueous extracts from different parts of the plant did not have any specific effect on *Ganoderma tropicum* and *Trichoderma koningi* molds. Among the tested molds, *Fusarium* showed the most sensitivity compared to other molds. They stated the reason for the different anti-fungal power in the extracts due to the different amount of effective phenolic compounds in aqueous, ethanolic and methanolic extracts. In another study conducted by Davoudi Moghadam et al. [11], the antifungal activity (*Aspergillus flavus* and *Aspergillus parasiticus*) of the essential oils of the mountain kakuti plant and its ability to inhibit corn aflatoxin were investigated. The results showed that the essential oils of the mountain kakuti plant were more effective on *Aspergillus parasiticus* than *Aspergillus flavus* in both types of laboratory tests.<sup>9</sup> and body conditions<sup>10</sup> Was. The essential oils of Kakuti plant showed the same minimum inhibitory concentration index against fungal species in the liquid culture medium, while these extracts showed different activities against *Aspergillus flavus* and *parasiticus* with minimum lethal concentration indices of 781.25 and 390.625 µg/ml, respectively. They showed themselves. Under corn storage conditions, the production of aflatoxin B<sub>1</sub> At concentrations of 6250 µg/ml for *Aspergillus flavus* and 6250 and 3125 µg/ml for *Aspergillus parasiticus*, it decreased significantly. At minimum concentrations, aflatoxin production increased gradually. Antifungal activity of essential oils extracted from fennel seeds was studied for use as a food

preservative. The results showed that the essential oils of fennel seeds had an inhibitory effect on the tested species (*Alternaria*, *Aerobasidium*, *Aspergillus fumigatus*, *Fusarium*, *Penicillium*, *Rhizopus* and *Trichophytonrubaro*). The dilution method was used to evaluate the indicators of minimum concentration of fungal inhibitor and minimum concentration of fungal killer. These indicators for fennel plant were between 625 and 1250 µg/ml. During this study, the antifungal index was also estimated and it was found that *Alternaria* was the most sensitive species [12]. The chemical composition and antibacterial activity of the essential oils of the mountain cockatoo were studied in laboratory conditions against some pathogenic bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Enterobacter aerogenes*). During this investigation, chemical compounds were obtained from the aerial parts of the mountain kakuti plant. The main ingredients include pulgone, piperitone, respectively<sup>11</sup>, 1-8-cineol<sup>12</sup>, neo-menthol<sup>13</sup>, mns-2-n-1-al<sup>14</sup> Menthol<sup>15</sup> Carvacrol<sup>16</sup> And Menton<sup>17</sup> They were. These oils had good activity against the tested bacteria except *Pseudomonas aeruginosa*, which is probably due to the high amount of polgon in their composition. Therefore, the essential oils of the mountain kakuti are a suitable medicinal plant against some human pathogenic bacteria [13]. Khaled et al.[14] studied the antifungal potential of fennel seed and leaf essential oils against phytopathogenic fungi (*Alternaria*, *Penicillium expansum*, *Rhizopus stolonifer*, *Fusarium* and *Aspergillus*). The analysis of essential oils obtained from both parts of the plant showed that trans-antol<sup>18</sup> And then Stragol<sup>19</sup> were the main ingredients. Antifungal activity of fennel seeds and leaves

9. In Vitro  
10. Live

11. Piperitenone  
12. 1-8- Cineole  
13. Neo-Menthol  
14. Menth-2-En-1-Ol  
15. Menthol  
16. Carvacrol  
17. Menthone  
18. Trans-Anethole  
19. Estragol

was investigated based on toxic food method and volatile compounds activity test against 5 pathogenic fungi. The results showed that the essential oils of fennel had the ability to prevent the growth of fungal mycelium species. Studies have shown that the volatile compounds test was much more sensitive than the toxic food method. Mycelial growth of *Alternaria*, *Fusarium* and *Aspergillus* fungi was completely inhibited by essential oils obtained from fennel leaves. The researchers stated that the most sensitive species to essential oils was *Rhizopus stolonifera*. In the current study, the antifungal activity of fennel and fennel seed extracts against *Aspergillus flavus* will be tested in tomato paste containing different percentages of the extracts. The innovation of the present study will be the use of artificial neural network in predicting the growth data of *Aspergillus flavus* mold in tomato paste with the number of intermediate layers (hidden) and different neurons. This neural network will be investigated with 2 inputs (extract concentration and storage time) and 1 output (*aspergillus flavus* mold growth). The evaluation of the investigated neural network will be done with the parameters of correlation coefficient, mean square error and maximum error.

## 2- Materials and methods

In this study, tomato paste, kakuti plant, and fennel seeds were obtained from Ik and Ik companies, University of Tehran and Zanjan region, respectively. *Aspergillus flavus* mold, Petto dextrose agar and Sabrod dextrose broth were purchased from Merck, Germany, respectively. Incubator, oven and bain marie (Memmert brand made in Germany) were also used. Other equipment such as desktop pH meter (model 3510 made by JENWAY UK), digital scale with (Sartorius brand with accuracy of 0.001 (Quintix124-1s model made in Germany), GC-MS machine (QP2010 SE Shimadzu model made in Japan), refractometer (ATAGO model MASTER-50H made in Japan), Rehan Teb autoclave made in Iran, colony counting machine (RTC made in China) and Behdad microbial hood made in Iran were used.

### 2-1- Preparation of alcoholic

### extracts of fennel seeds and mountain kakuti leaves

To prepare alcoholic extracts of fennel seeds<sup>20</sup> and the mountain kakuti plant<sup>21</sup> A grinder with 40 mesh was used. First, the necessary amount (about 30 grams) of the powder of the prepared samples of fennel seed and kakuti plant was poured into separate jars and then each of the samples was mixed with 100 ml of 70 degree ethanol (with a ratio of 1 to 3). The container containing the samples is individually placed on the magnetic stirrer so that the samples are completely mixed with each other. After the samples were completely mixed, they were filtered by Whatman 42 paper, and the resulting solution was concentrated with a rotary evaporator under vacuum (Rotary Evaporator made by Heidolph). Germany) at a temperature of 50 degrees Celsius so as not to damage the phenolic compounds. Then the extract obtained was filtered for one hour with a 0.25 micron Millipore syringe filter and kept in a dark and sterilized container until use.] 6 [ Figures 1 and 2 show tomato paste with different concentrations of fennel seed and mountain kakuti leaf extracts.

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20. *Feniculum Vulgare* Mill

21. They are *Clinopodioides* Lam



**Fig 1** Samples of tomato paste with different concentrations of *Foeniculum vulgare* Mill extract



**Fig 2** Samples of tomato paste with different concentrations of *Ziziphora clinopodioides* Lam extract

## 2-2- Preparation of samples

For the preparation of spore suspension, *Aspergillus flavus* mold prepared from the mycology department of the Faculty of Veterinary Medicine of Tehran University was used. First, the mold in Potato dextrose agar broth medium<sup>22</sup> The slant was kept in a greenhouse for 7-10 days at a temperature of  $25 \pm 5^\circ\text{C}$  to produce spores. 10 ml of 0.05% Tween 80 solution was added and the culture surface was gently scratched with a sterile bent glass rod to collect spores. In order to remove mycelium parts, the suspension was filtered using glass wool. The number of spores was counted by hemocytometer and the concentration of spores was determined by 0.05 Tween 80 solution.<sup>5</sup> 10 spores per milliliter were reached [15]. To determine<sup>23</sup> MIC (minimum inhibitory concentration) was used to determine the wavelength, which was carried out by a spectrophotometer optical wavelength absorption device (QP2010 SE Shimadzu made in Japan). First, Nim McFarland solution was poured into the spectrophotometer cell and the absorbance at the wavelength of 620 nm should be between 0.08 and 0.13, in which case the mold count is  $10^{8 \times}$ . It shows 1.5 was accepted. To get *Aspergillus flavus* mold it up to  $10^5$  Must be diluted [16]. For this purpose, the desired amount of mold was first read using the half

McFarland wavelength in a liquid culture medium such as Sabrose broth, and using a tube containing a mold suspension in several dilution tubes, and from these dilutions, the desired amount (cfu) was used to inoculate the bacteria.  $/\text{ml}10^5$ , was used [6]. To inoculate the samples with 100 grams of tomato paste (one and one) pasteurized at  $85^\circ\text{C}$  for 20 minutes with  $\text{pH} = 4.3$  and then extracts of fennel seed and Millipore 25/25 filter. 0 micron was sterilized and added to tomato paste with concentrations of 2, 1, 0.5 and 3% in completely sterile conditions in separate containers, and from *Aspergillus flavus* mold suspension with a final concentration of  $10 \text{ ml}/\text{cfu}^5$  0.1 ml was added and finally they were kept in an incubator (Memmert brand, made in Germany) at a temperature of  $25^\circ\text{C}$  for 35 days, and microbial culture was done once a week to check the growth rate [6]. At the time of microbial culture from tomato paste sample to dilution  $3^{-10}$  with Potato dextrose agarbe broth culture medium amount of 14 cc was poured in each plate, then it was kept next to the flame and in completely sterile conditions after closing the gel in the incubator at a temperature of  $25 \pm 0.5$  degrees Celsius and after 7 days the plates was taken out of the incubator and the molds were counted. Mold activity was measured using agar dilution method. The dilution solution contains peptone and sodium salt, which was finally examined with an electron microscope. The analysis was performed as follows with 3 repetitions. The

22. Potato Dextrose Agar

23. Minimum Inhibitory Concentration



medium (in vitro) consisted of tomato paste, fennel seed extracts and mountain cockatoo with different concentrations along with *Aspergillus* mold spores, whose culture medium is Petto dextrose agar broth. Then by injecting 0.1 ml of mold in Sabrod Dextrose Agar broth culture medium<sup>24</sup> and placed in an incubator at a temperature of  $25 \pm 0.5^\circ\text{C}$  for 5 weeks (35 days) where a culture was performed every week to check the activity of mold in different concentrations of the extracts. At the time of microbial culture, 1 ml of liquid with mold spores containing different concentrations of fennel and fennel seed extracts was removed, and then 14 cc of dextrose agar was poured into each plate and next to the flame in completely sterile conditions. After closing the gel, it was kept in an incubator at a temperature of  $25 \pm 0.5^\circ\text{C}$ , and after 7 days, the plates were taken out of the incubator and the molds were counted by dilution method and with an electron microscope [5].

### 3- Artificial neural networks

Artificial neural network<sup>25</sup> which is inspired by the biological nervous system and deals with information processing. This system consists of a large number of highly interconnected processing elements called neurons that work together to solve a problem. Using learning algorithms, this network collects information from its surroundings and then prepares them for exploitation. There is a relationship between the output of each neuron and the inputs of other neurons, which is defined by a certain weight according to the effect of the output of the neuron on the output of other neurons. By using weights, storage is actually done. One of the types of neural networks can be perceptron neural network<sup>26</sup> Cited. A perceptron takes a vector of inputs with real values and calculates a linear combination of these inputs. If the result of these calculations exceeds a threshold value, the output of the perceptron will be equal to one, otherwise it will be equal to negative one. Perceptron neural network evaluation indices

with Levenberg-Marquadt algorithm<sup>27</sup> It works is the correlation coefficient and the mean squared error which is expressed in the present study according to the following equations [17].

$$R^2 = \left( 1 - \frac{\left[ \left( \sum_{i=0}^n (e_{i(Exp)} - e_{i(Ann)}) \right)^2 \right]}{\sum_{k=0}^n (e_{Exp})^2} \right) \quad (1)$$

$$\text{MSE (Mean Squared Error)} = \frac{1}{N} \sum_{i=1}^N (e_{i(Exp)} - e_{i(Ann)})^2 \quad (2)$$

In Eq<sub>s</sub>  $e_{i(Ann)}$ ,  $e_{i(Exp)}$ , MSE,  $R^2$  Correlation coefficient, mean square error, laboratory data values and results obtained by artificial neural network are respectively. Figure 3 shows the general schematic of the neural network used in this study.

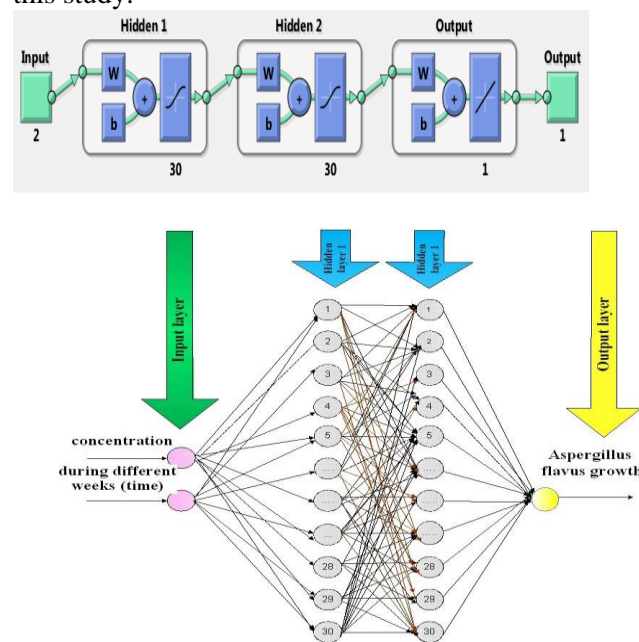


Fig 3 Neural network with two hidden layers with 30 neurons in each layer

## 4- Discussion and conclusion

### 4-1- Identification of the compounds in the extracts of fennel and fennel seeds

To identify the compounds of the extracts, they were first prepared for injection into the GC-mass machine and injected into the machine. Figures 4 and 5 show the chromatograms of

24. Sabouraud Dextrose Broth

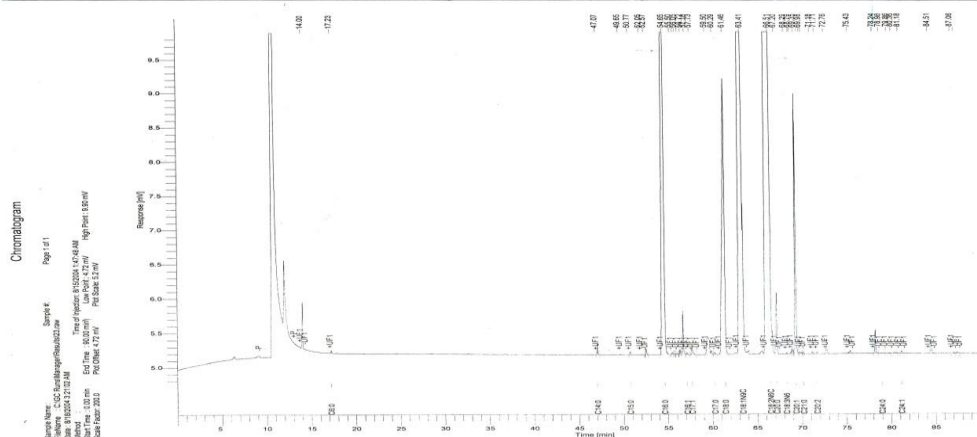
25. Artificial Neural Networks

26. Perceptron

27. Levenberg-Marquardt

fennel seed extract and mountain kakuti. Tables 1 and 2 are the analysis of compounds of fennel seed extract and fennel seed extract. The purpose of analyzing the composition of extracts

is to check their structure, whether it can act as a preservative in preventing the growth of mold or reduce its growth.



1.64	1,8-cineole	4.12	n-nonadecane
1.52	piperitone	4	n-octadecane
0.98	the gamma terpins	3.98	hexadecane
0.95	carvone	3.7	n-heptadecane
96.3	Total identified	0.84	pentadecane

## 2-4- Investigating the growth of *Aspergillus flavus* mold in tomato paste containing different concentrations of fennel seed extracts and mountain cockatoo in vitro

The results of the effect of different concentrations of fennel seed extract and mountain cockatoo extract on the growth rate of *Aspergillus flavus* mold in the environment (in vitro) are shown in Table 3 and Figure 6. As shown in Table 3, the growth of *Aspergillus flavus* mold in the medium of tomato paste containing different percentages of two types of extracts depended on the concentration of the extracts and the storage time of the samples. In Duncan's spss output, the groups have equal averages, the groups with 2% fennel extract and 3% fennel extract are equal to each other, the groups with 1% fennel extract and 3% fennel extract are equal to each other, the groups with 1% fennel extract and fennel extract are equal to each other. 2% kahi are equal to each other, groups with 0.5% fennel extract and 1% and 2% kakuti extract are equal to each other, groups with 1% and 0.5% kakuti extract are equal to each other (Table 4). It can be seen that with the increase in the percentage of fennel and fennel extracts, the percentage of mold decreases, although in the groups with 2 and 3% fennel extract, the average was zero, which are the best groups in terms of the absence of mold. During the 5-week storage period, no moldy mycelium was seen on them and it was repeated three times and they showed resistance to mold growth and prevented the growth of *Aspergillus flavus* mold. Minimum bactericidal

concentration<sup>28</sup>(MBC) in fennel seed extract in 3 and 2% concentration. In the environment without tomato paste, the growth of mold in the liquid medium in different concentrations of extracts also stops the growth of mold in 2 and 3% of fennel seeds.

The antimicrobial activity of various compounds found in plant extracts and essential oils, especially phenolic compounds, has been reported in various articles. Khaled et al.[14] evaluated the effect of using fennel seed essential oil on different mold pathogens. These researchers stated that the use of fennel seed essential oil is able to prevent the growth of mycelia of all studied molds. Davoudi Moghadam et al.[11] studied the effect of using the essential oil of mountain kakuti to prevent the production of aflatoxin by *Aspergillus flavus* and *Aspergillus parasiticus*.

28. Minimum Bacteriocidal Concentration



**Table 3** *Aspergillus flavus* growth variations in terms of log cfu / ml in tomato paste containing *Foeniculum Vulgare* Mill and *ziziphora clinopodioides* Lam. extracts in vitro

Aspergillus flavus growth variations during different weeks					
Fifth week	Fourth week	Third week	Second week	First week	Sample
0.19 <sup>Aa</sup> ±6.992	0.16 <sup>Bb</sup> ±6.608	0.42 <sup>Bb</sup> ±6.107	0.09 <sup>Ab</sup> ±5.600	0.00 <sup>Ad</sup> ±5.000	Control
0.07 <sup>Ab</sup> ±5.192	0.57 <sup>Ab</sup> ±4.431	0.058 <sup>Ab</sup> ±4.303	1.15 <sup>Ab</sup> ±3.676	1.00 <sup>And</sup> ±3.213	Treatment 1
0.49 <sup>Ab</sup> ±4.819	0.58 <sup>Ab</sup> ±4.192	0.99 <sup>Ab</sup> ±3.310	1.10 <sup>Ab</sup> ±2.567	1.00 <sup>Ab</sup> ±1.104	Treatment 2
0.007 <sup>Cb</sup> ±0.00	0.00 <sup>Cb</sup> ±0.00	0.00 <sup>Cb</sup> ±0.00	0.00 <sup>Cb</sup> ±0.00	0.00 <sup>Cb</sup> ±0.00	Treatment 3
0.00 <sup>Cb</sup> ±0.00	0.00 <sup>Cb</sup> ±0.00	0.00 <sup>Cb</sup> ±0.00	0.00 <sup>Cb</sup> ±0.00	0.00 <sup>Cb</sup> ±0.00	Treatment 4
0.00 <sup>Aa</sup> ±6.626	0.00 <sup>Bb</sup> ±5.566	0.58 <sup>Ab</sup> ±4.933	0.00 <sup>Ab</sup> ±4.426	0.00 <sup>Ad</sup> ±4.266	Treatment 5
0.00 <sup>Bb</sup> ±5.517	0.00 <sup>Bb</sup> ±5.253	0.05 <sup>Ab</sup> ±4.293	0.56 <sup>Ab</sup> ±3.873	0.58 <sup>And</sup> ±3.459	Treatment 6
0.00 <sup>Fb</sup> ±5.121	0.00 <sup>Db</sup> ±4.666	0.52 <sup>Cb</sup> ±3.923	0.00 <sup>Bb</sup> ±3.033	0.06 <sup>Ab</sup> ±2.185	Treatment 7
0.52 <sup>Fb</sup> ±4.982	0.58 <sup>Db</sup> ±3.899	0.17 <sup>Cb</sup> ±3.024	0.00 <sup>Bb</sup> ±2.133	0.02 <sup>Of</sup> ±1.130	Treatment 8

\* Different capital letters in a row represent significant difference (p < 0.05).

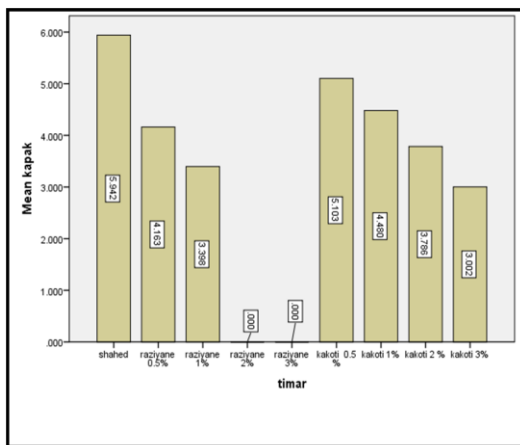
\* Different small letters in a column represent significant difference (p < 0.05).

Treatment 1 (containing 0.5% fennel seed extract), treatment 2 (containing 1% fennel seed extract), treatment 3 (containing 2% fennel seed extract), treatment 4 (containing 3% fennel seed extract), treatment 5 (containing 0.5% *ziziphora clinopodioides* Lam. extract), treatment 6 (containing 1% *ziziphora clinopodioides* Lam. extract), treatment 7 (containing 2% *ziziphora clinopodioides* Lam. extract) and treatment 8 (containing 3% *ziziphora clinopodioides* Lam. extract).

**Table 4** Variance analysis of *Aspergillus flavus* growth

P	F	Mean squares	Degree of freedom	Source variation
*0.000	84.997	16.790	4	Storage time (A)
*0.000	327.300	64.655	8	Sample type (B)
*0.000	4.210	0.832	32	Interaction (A×B)
	R-Sq (R <sup>2</sup> )	95.8%		

\*Significant difference at probability level 5%

**Fig 6** Growth changes of *Aspergillus flavus* mold in terms of log cfu/ml in tomato paste and different concentrations of *Foeniculum Vulgare* Mill and *ziziphora clinopodioides* Lam. extracts during storage in vitro

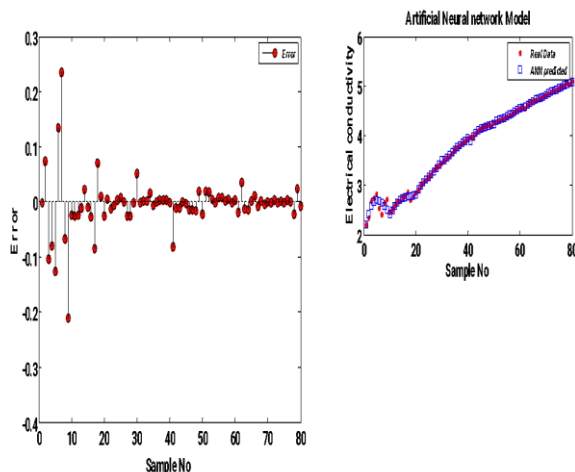
The results of the study of these researchers showed that the use of Kakuti essential oil prevented the growth of both molds, but its effect was greater on *Aspergillus parasiticus*.

Also, the use of this essential oil effectively reduced the production of corn aflatoxin by the two mentioned molds. Noshirvani et al. [19] evaluated the anti-mold and anti-oxidant effects of fennel plant extract. Their results showed that fennel extract has free radical scavenging activity, especially in high concentrations<sup>29</sup> had high DPPH, although its effect was less than<sup>30</sup> It was TBHQ. The results of oxidation activity showed that fennel extract and powder reduced the rate of oxidation of sunflower seed oil compared to the control sample. Based on the obtained results, fennel extract can be used as a healthy plant source with good anti-oxidation and anti-mold properties. Therefore, it can be concluded that the presence of active compounds such as antole, pulgon and thymol in both extracts is responsible for their anti-mold activity in tomato paste. As seen in Figure 7, the

29. 2,2-Diphenyl-1-picrylhydrazyl

30. Tert-Butylhydroquinone

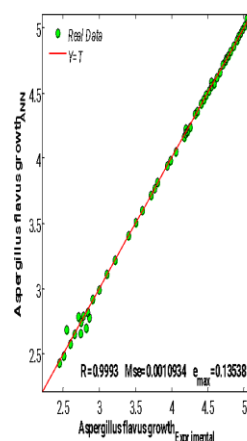
obtained error values are scattered around the zero value. The highest and lowest error values are between 0.25 and -0.2. Most of the values obtained from the network have an error value of zero and the highest error value is in samples 1 to 10.



**Fig 7** The values obtained by error with the number of samples

The more the dispersion of the data is on the zero line, the better the result and the more successful the network is in prediction [17-18]. Figure 8 shows a comparison between experimental data and predicted growth of *Aspergillus flavus* mold by artificial neural network with 30 neurons in each hidden layer. As can be seen, the data are scattered around the 45 degree line, and the more these points are on the 45 degree line, the higher the correlation coefficient is and is close to 1. The obtained values of correlation coefficient, mean square error and maximum error are equal to 0.9993, 0.0010934 and 0.13538 respectively. The results of this prediction are in good agreement with the results obtained from Mokhtarian et al.'s study [20]. They used perceptron neural network to predict humidity and speed ratio.dry They used tomato slices. The best arrangement of the neural network for the first network is based on one hidden layer, 2 and 8 neurons in the hidden layer, respectively, for humidity and sound ratio.dryWas. The values of the correlation coefficient and the obtained error value are equal to 0.999 and 33.43, respectively. The effectiveness of using neural network was investigated by Hashem Lo et al. [21] in

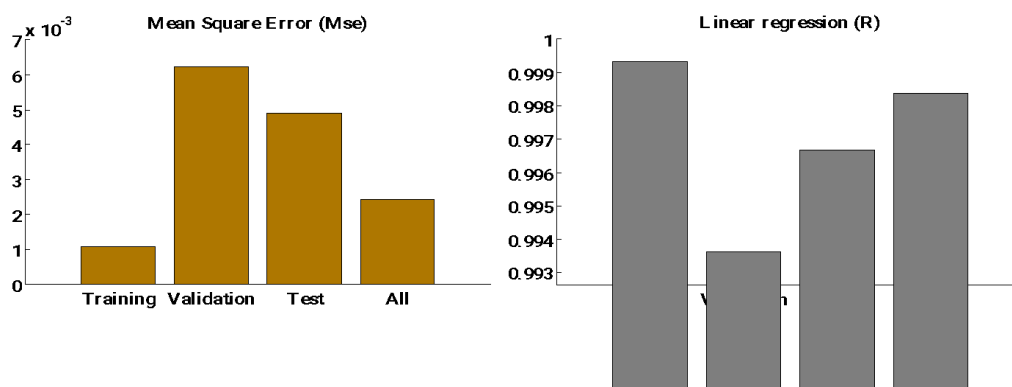
modeling the operation of frying dried tomatoes in different osmotic solutions (sugar and salt) with different concentrations and sesame and sunflower oils at different temperatures and times. .



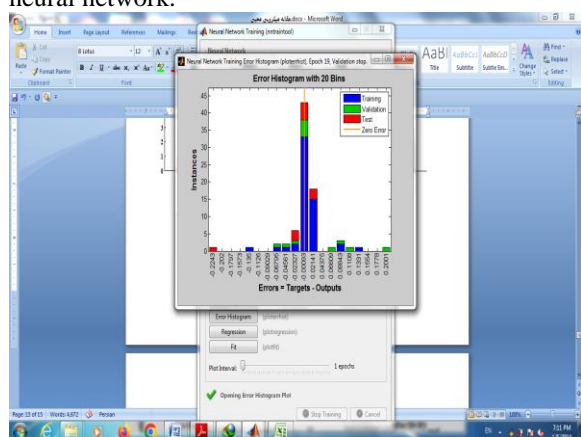
**Fig 8** Comparison of Experimental values and artificial neural network of *Aspergillus flavus* mold growth

The inputs used in neural networks included drying temperature, frying temperature, type of oil, and concentration of osmotic solution, and the outputs included moisture content and sample color values L, a, b. The results showed that a network with two hidden layers and 5 neurons in each layer has the best result for predicting the moisture value and L value. In general, the results of this study showed that the perceptron artificial neural network model as an efficient tool can estimate the basic parameters of the frying process with high accuracy.

Figure 9 shows the exact values of correlation coefficient and mean square error for training, validation, test and total data. 70% of the total data is allocated to training data. As can be seen, the values of correlation coefficient and mean square error have values of 0.9995 and 0.001, respectively. These values are also clearly shown for the validation and test data. In Figure 10, the horizontal axis shows the difference between the experimental and predicted data and the vertical axis shows the number of samples. The error dispersion is in the range of  $\pm 0.2$ , which indicates a successful prediction with the 1-30-30-2 topology [22].



**Fig 9** The values obtained are the mean squared error and the correlation coefficient of the data with the artificial neural network.



**Fig 10** Histogram of the error obtained with the number of samples examined

## 5. Conclusion

The antimicrobial properties of herbal products have been of public interest for many years, and among these products, essential oils and plant extracts have been highly regarded as natural preservatives in recent years, and many studies by food industry researchers have focused on the use of these compounds. The aim of this study is the effect of fennel seed extracts and mountain cockatoo on the growth of *Aspergillus flavus* mold in tomato paste and finally the results were evaluated by perceptron neural network. The results of the antifungal activity of the extracts in tomato paste showed that the growth rate of *Aspergillus flavus* mold was limited by increasing the percentage of the extracts, and generally after the end of the storage period (after 5 weeks of storage) the treatments containing 2 and 3% of fennel seed extract showed no mycelium growth. *Aspergillus*

*flavus* mold was not observed on its surface. The evaluation results predicted by artificial neural network with 1-30-30-2 topology (with two inputs, two hidden layers with 30 neurons in each layer and one output) show that the obtained values of correlation coefficient, mean squared error and maximum error are The order is equal to 0.9993, 0.0010934 and 0.13538, which indicates a successful prediction.

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## بررسی تأثیر عصاره‌های دانه رازیانه و کاکوتی کوهی روی رشد کپک آسپرژیلوس فلاووس رب گوجه فرنگی و پیش‌بینی داده‌های حاصل شده با استفاده از شبکه‌های عصبی مصنوعی

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اطلاعات مقاله	چکیده
<p>تاریخ های مقاله :</p> <p>تاریخ دریافت: ۱۴۰۱/۰۳/۲۱</p> <p>تاریخ پذیرش: ۱۴۰۱/۰۹/۲۷</p>	<p>فعالیت ضد قارچی عصاره‌های دانه رازیانه و کاکوتی کوهی علیه آسپرژیلوس فلاووس در رب گوجه‌فرنگی حاوی درصد‌های مختلف از عصاره‌ها مورد آزمون قرار گرفت. برای این منظور عصاره‌های دانه رازیانه و کاکوتی کوهی با غلظت‌های متفاوت ۰/۵، ۱ و ۲ درصد تهیه گردید و در مدت زمان نگهداری ۳۵ روز بررسی شد. تأثیر عصاره‌های دانه رازیانه و کاکوتی کوهی با غلظت‌های مختلف به تنهایی در محیط (in vitro) بررسی گردید. با تزریق کپک ۰/۱ میلی‌لیتر در محیط کشت آبگوشت ساپرود دکستروز آگار<sup>۱</sup> و سپس قرار دادن در دمای انکوباتور ۲۵ ± ۰/۵ درجه سانتی گراد به مدت ۵ هفته (۳۵ روز) نگهداری شد که هر هفته یک کشت انجام گرفت تا فعالیت کپک در غلظت‌های مختلف عصاره‌ها بررسی گردد. نتایج فعالیت ضد قارچی سطوح مختلف عصاره‌ها نشان داد که تیمارهای ۳ (حاوی ۲ درصد عصاره دانه رازیانه) و ۴ (حاوی ۳ درصد عصاره دانه رازیانه) تا پایان دوره نگهداری در مقابل رشد میسلیوم‌های کپک آسپرژیلوس فلاووس مقاوم بودند. به‌طور کلی می‌توان نتیجه گرفت که استفاده از ۲ یا ۳ درصد عصاره دانه رازیانه به‌عنوان نگهدارنده طبیعی در رب گوجه‌فرنگی فعالیت ضد قارچی مطلوبی دارند. برای صحت‌سنجی و ارزیابی نتایج حاصله از آزمایشات از شبکه عصبی مصنوعی در پیش‌بینی داده‌های رشد کپک آسپرژیلوس فلاووس رب گوجه‌فرنگی استفاده گردید. در این بررسی از تعداد دولایه مخفی با تعداد ۳۰ نرون استفاده شد. شبکه دارای دو ورودی غلظت عصاره و مدت زمان نگهداری بوده و رشد کپک آسپرژیلوس فلاووس به‌عنوان تارگت در نظر گرفته شد. پارامترهای ارزیابی از قبیل ضریب همبستگی، میانگین مربعات خطا و ماکزیمم خطا نتایج بسیار مطلوبی با مقادیر ۰/۹۹۹۳، ۰/۱۰۹۳۴ و ۰/۱۳۵۳۸ را نشان دادند. هر چه مقدار خطا کمتر باشد و میزان ضریب همبستگی نزدیک یک باشد نشان از یک پیش‌بینی مطلوب است.</p>
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