Journal of Mycology Research. Vol 2, No 1, March 2015, Page 23-30

Effect of savory essential oil, garlic powder, and garlic aqueous extract on fungal load of poultry feed

Abdolghaffar Ownagh[*1]; Mahnaz Fallahi[2]; Bentolhoda Rahman[3]and Davood Mohammadzadeh[2]

[1]Associate Professor of Microbiology Department, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[2]Graduate of Veterinary Medicine, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[3]PhD student in microbiology, Faculty of Veterinary Medicine, Urmia University,Urmia, Iran

*** Corresponding Author: E-mail: ownagh@yahoo.com; Tel: +98 4432770508**

(Received: 1 April 2015, Accepted:28 April 2015)

Abstract:

In this study, we investigated the effect of savory (*Satureja hortensis*) essential oil, garlic (*Allium sativum*) powder, and garlic aqueous extract on fungal load of poultry feed. To this end, savory essential oil and garlic aqueous extract each amounting 62.5, 125, and 250 µl/ml and mg/ml, respectively, per kilogram of feed and garlic powder amounting 2.5, 5, and 10 g/kg of feed were used. One and two weeks after adding the desired amounts of these compounds to poultry feed, old counting was carried out at 2, 24, 48, and 72 h. Then, the results of mold counting per gram of feed were evaluated in the treatment groups compared to the control group. Based on results, fungal load of feed was reduced 2 h after adding savory essential oil, and this effect was sustained until the end of the second week. This effect was concentration-dependent, and hence fungal load reached to zero at the highest concentration (250 µl/ml) 48 and 72 h after adding savory essential oil ($P< 0/05$). Garlic powder significantly reduced the fungal load of feed, especially in the treatment group containing the maximum dose (10 g) 72 h after adding garlic powder ($P< 0.05$). The garlic aqueous extract increased fungal load of feed considerably ($P< 0.05$). So, it is suggested that savory essential oil and garlic powder can be used as appropriate alternatives for chemical agents used in controlling fungal load of poultry feed.

Keywords: feed, fungal load, garlic powder, poultry, savory essential oil.

Introduction

As an important source of human food supply, the poultry industry has a significant role in providing healthy nutrition. Obviously, in order to produce healthy food with poultry origin, it is necessary for components of poultry feed to be safe and of good quality. The presence of microorganisms in poultry feed leads to the corruption and reduction in nutritional value (Shareef, 2010), incidence of acute and contagious diseases, allergy and poisoning (Ariyo *et al*., 2013). It can cause extensive economic losses and high mortality (Krnjaja *et al*., 2008). One of the microorganisms that infect poultry feed components are fungi (Krnjaja *et al*., 2008; Ariyo *et al*., 2013). The presence of fungi and their toxic metabolites

(mycotoxins) in poultry diets, especially in tropical areas, is unavoidable problem (Aly and Anwer, 2009). Different species of fungi such as *Fusarium*, *Aspergillus*, *Mucor*, and *Penicillium* spp. have been isolated from poultry diets (Moreno Romo and Fernandez, 1986). *Aspergillus* species, one of the main contaminants of poultry diets, produce aflatoxins (Aly and Anwer, 2009). Moderate or high levels of aflatoxin in the diet can lead to mortality, while low levels of these toxins reduce the production indices. The food originating from poultry containing aflatoxins is a significant threat to consumer health and consequently many studies have confirmed carcinogenicity of these toxins for humans (Hsieh and Atkinson, 1991; Binder *et al*., 2007). Hence, management and control of fungal pollution, especially mycotoxins, is of utmost importance in order to reduce their harmful effects.

The use of disinfectants to control feed contamination is limited due to the sensitivity of poultry feed ingredients. Conventional methods for microbial control of food such as steaming plate, use of ozone and formaldehyde gases, radiation, organic acids such as formic acid and propionic acid and methyl bromide are often associated with undesirable side effects, including toxic effects of some remnants of these compounds, such as reproductive disorders and reduced fertility, behavioral disorders, tumorigenesis, and changes in Complete Blood Cell (CBC) (Khan *et al*., 2006). On the contrary, these methods do not be effective in the control of microbial load completely. Considering what was mentioned, attempts to find alternatives with fewer side effects and greater efficiency to control microbial load in poultry feed seem absolutely necessary.

In recent years, the use of herbal medicines and natural products, due to antimicrobial properties and minimal side effects, is rising. Garlic (*Allium sativum*) and savory (*Satureja hortensis*) herbs have consumption history of thousands of years, and their antifungal properties have been confirmed according to many studies (Irkin and Korukluoglu, 2007; Razzaghi-Abyaneh *et al*., 2008; Fani and Araghizadeh, 2009; Yazdanpanah Goharrizi *et al*., 2012). In this study, we evaluate the effect of savory essential oil, garlic aqueous extract, and garlic powder on fungal load of poultry feed.

Materials and Methods

Preparation of savory essential oil: Savory herb was obtained from agriculture college greenhouse of Urmia University and dried in shade. About 100 g of dried aerial parts were subjected to hydro-distillation by Clevenger apparatus for 3 h. The savory essential oil was dried over anhydrous sodium sulfate and stored at 4°C until use (Yazdanpanah and Mohamadi, 2014). The normal saline was used for preparation of 62.5, 125, and 250 µl/ml concentrations.

Preparation of garlic aqueous extract: About 80 g of fresh garlic bulbs were surface sterilized with 1.0% sodium hypochlorite (NaOCl) and grounded in a sterile mixer. The pounded garlic was mixed with 100 ml of sterile distilled water. The mixture was centrifuged at 6000 rpm for 20 min. The supernatant was filtered through Whatman No. 1 paper and then through 0.45 µm membrane filters. The active ingredient in the extract was 512 mg/ml (Bakri and Douglas, 2005). Strile distilled water were used for preparing 62.5, 125, and 250 mg/ml doses of the garlic aqueous extract. The extract was stored in a refrigerator (4 °C).

Preparation of garlic powder: Alliums were peeled, washed with sterile distilled water, and crushed. Then, the cloves were dried in

oven at 50 °C for 48–72 h. The dried garlic was grounded in a blender and resulting powder was passed through sieve of 1 mm mesh size. The garlic powder was placed into sterile containers and stored at 4°C (Khan and Zahoor, 2014).

Preparation of samples: Four broiler farms around Urmia city were selected randomly. A typical 3 kg of ready-for-consumption poultry feed was collected in sterile plastic containers from mixer device of each farm. Collected samples were transferred in compliance with the standard conditions to the microbiology laboratory, faculty of Veterinary Medicine, Urmia University. The samples were thoroughly mixed and were divided into ten 1 kg samples in sterile plastic containers. About 5 ml of savory essential oil with concentrations of 62.5, 125, and 250 µl/ml was sprayed to each kg of feed with complete uniformity and under sterile conditions. About 2.5, 5, and 10 g of garlic powder per kilogram of feed was added to the samples and mixed well by a clean mixer. Also, five ml of garlic aqueous extract with different concentrations of 62.5, 125, and 250 mg/ml in sterile condition was sprayed uniformly to each kilogram of feed. All treatment groups consisted of three replicates.

Fungal count: Fungal count was performed according to the method described by Ariyo *et al*. (2013). One and two weeks after mixing of poultry feed with savory essential oil, garlic powder, and garlic aqueous extract, the mold count was carried out at 2, 24, 48, and 72h. During the study, treated and control samples of feed were kept in feed warehouse in hennery. Finally, results of mold count per gram of feed in treated groups compared to control group were evaluated.

Statistical analysis

The results were analyzed using Minitab software, and the data were studied as mean ± SD (Confidence level 95%).

Results

Count of fungal colonies showed reduction in fungal load in a concentration-dependent manner 2 h after adding savory essential oil. This effect lasted up to two weeks. The most antifungal effect was observed 48 and 72 h after adding oil, and therefore the fungal load of feed reached to zero ($P < 0.05$) in treatments containing 125 and 250 µl/ml savory essential oil. Also, 24 h after adding garlic powder, fungal load of feed was significantly reduced compared to the control group ($P < 0.05$). Like savory essential oil, antifungal effect of garlic powder was dose-dependent and lasted till the end of second week. The maximum antifungal activity was observed in the treatments containing 10 g of garlic powder and after 72 h ($P < 0.05$). Based on the results of fungal count in treatments receiving garlic aqueous extract, this extract increased fungal load of feed significantly ($P <0.05$) compared to control group. The results of mold count after addition of savory essential oil, garlic powder, and garlic aqueous extract to feed poultry are shown in Figure 1 to 3.

Discussion

Molds are important contaminants of poultry feed that produce mycotoxins and can also cause corruption of food. According to studies, 30% to 40% of molds can produce toxic compounds in favorable conditions (Shareef, 2010). Most toxic species belong to the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* (Kaushal and

Sinha, 1993). Mycotoxicosis in poultry flock leads to reduction in weight gain, egg production and shell quality, anorexia, increase of eggs with blood spots, increased mortality, immunosuppression, failure of vaccination programs, susceptibility to infectious diseases such as Colibacillosis and Newcastle, and many other problems (Shareef, 2010). The consumption of meat, egg, and poultry viscera containing the remains of mycotoxins leads to transmission of these toxins to humans (Pennington, 1986; Naoom, 2007). Therefore, control of hygiene and quality of poultry feed is very important for public health.

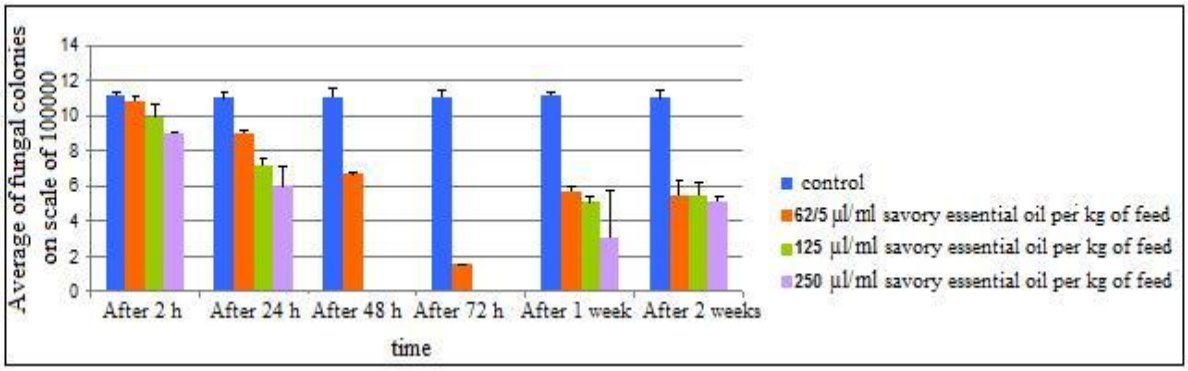

Fig. 1. Results of mold count during two weeks after adding savory essential oil to feed poultry

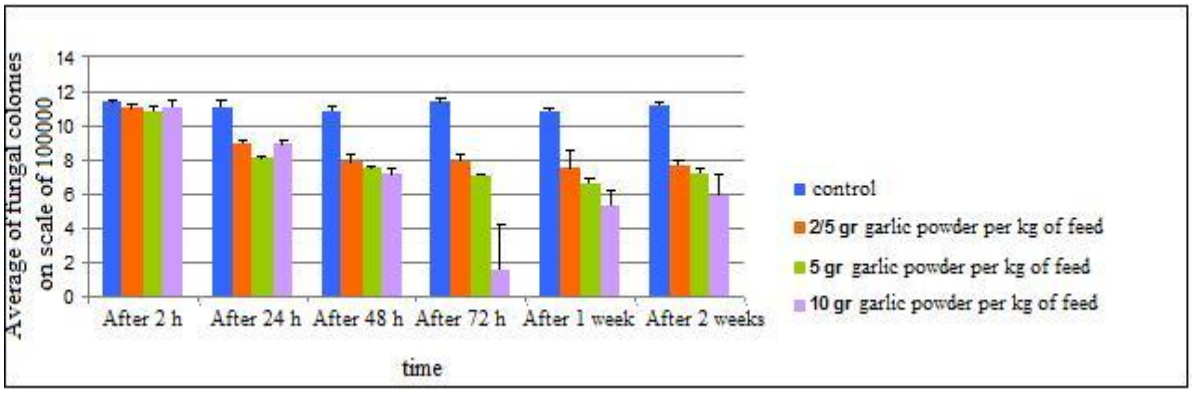

Fig. 2. Results of mold count during two weeks after adding garlic powder to feed poultry

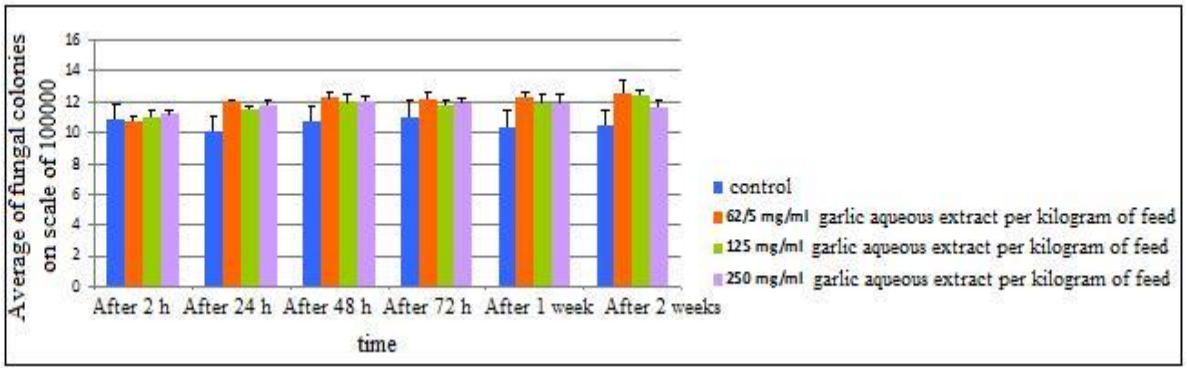

Fig. 3. Results of mold count during two weeks after adding garlic aqueous extract to feed poultry

The use of disinfectants to control poultry feed contamination has always been accompanied with restrictions due to the sensitivity of food components. Also, some of these methods have various side effects. For example, high concentrations of

formalin in poultry feed are associated with adverse effects as depression, anorexia, anemia, leukopenia, and an increase in blood urea and creatinine levels (Khan *et al*., 2006). These problems have caused to raise efforts to find the effective herbal products with minimal side effects increasingly.

In this study, we investigated antifungal properties of savory essential oil, garlic powder, and garlic aqueous extract on fungal load of poultry feed. Antifungal property of these herbal products has been recorded in previous studies (Adiguzel *et al*., 2007; Borhan-Mojabi *et al*., 2012; Mercy *et al*., 2014; Yazdanpanah and Mohamadi, 2014). According to the results, 2 h after adding savory essential oil, fungal load of feed was reduced in a dose-dependent manner. The maximum effect on fungal load was observed for savory essential oil concentrations of 125 and 250 µl/ml during 48 and 72 h after adding oil, and therefore no fungal colonies were observed on plates. These results are in agreement with the results of other studies, where strong antifungal activity of savory essential oil against *Candida albicans* (Majd et al., 2009; Mohammadpour et al., 2011), *Alternaria citri* (Yazdanpanah and Mohamadi, 2014), and *Aspergillus flavus* and inhibition of aflatoxin B1 production (Yazdanpanah Goharrizi et al., 2012) were approved previously. Evaluation of the antimicrobial properties of savory essential oil by Sahin et al. (2003) showed that the oil significantly reduced the microbial load of feed. Furthermore, it was found that savory essential oil has antioxidant properties, and it can be used for replacement of synthetic antioxidant compounds in poultry diet (Montazeri *et al*., 2014).

Antifungal effect of garlic powder was observed 24 h after adding it to feed. Antifungal activity of garlic powder was lower than savory essential oil. The most antifungal effect was reported in 72 h after adding 10 g of garlic powder per kilogram of feed. Antifungal properties of garlic are confirmed in previous studies (Lemar *et al*., 2002; Low *et al*., 2008; Londhe *et al*., 2011; Khan and Zahoor, 2014). In order to maintain the quality of poultry feed, the antifungal activity of garlic powder has already been investigated (Khan and Zahoor, 2014). In this study, *Aspergillus flavus* and *Aspergillus parasiticus* were added to the feed containing garlic powder. The results showed strong antifungal properties of garlic in which the growth of these fungi was inhibited in food treated with garlic powder. Sallam *et al*. (2004) studied the antioxidant and antimicrobial effects of garlic in sausage. The results showed that garlic has strong antimicrobial and antioxidant properties, and hence it can be used as an additive for preserving foodstuffs. In the study conducted by Berges *et al*. (2004), it was found that garlic reduces carcinogenicity of cancer-causing chemical compounds, especially aflatoxin B1, in rats fed with diet containing this toxin; they found that garlic increases the enzymes responsible for detoxification of aflatoxin B1. Several studies have indicated that use of garlic in poultry diets, in addition to its antimicrobial properties, has positive effects on poultry health and production (Qureshi *et al*., 1983; Konjufca *et al*., 1997; Olobatoke and Mulugeta, 2011; Reeisi *et al*., 2012).

Garlic aqueous extract increased fungal load of feed 2 h after adding to diet. Shamim *et al*. (2004) examined the effect of aqueous and alcoholic extract of garlic on various species of Dermatophytes, Saprophytes, and *Candida* spp. Results indicated that the antifungal activity of ethanol extract of garlic was considerably higher than aqueous extract (Shamim *et al*., 2004). Also, the study conducted by Mercy *et al*., (2014)

showed that the antifungal activity of ethanol extract of garlic against dermatophytes was more than its aqueous extract. However, evaluating the antifungal effect of aqueous extract of garlic and onion on different species of *Candida* and *Malassezia furfur* revealed that these compounds have good antifungal properties (Shams-Ghahfarokhi *et al*., 2006). In another study, the effect of garlic aqueous extract on pathogenic yeasts was reported similar to ketoconazole (Razaghparast *et al*., 2009). Investigate by Irkin and Korukluoglu (2007) also confirmed the inhibitory effects of garlic aqueous extract against *Aspergillus niger*. Stronger antifungal effects of garlic ethanol extract compared with its aqueous extract have been attributed to the solvent used in extraction of the active ingredients of garlic. Given that ethanol and methanol are organic solvents, their solubility is more than water for dissolving the active ingredient of garlic required for antifungal activity (Mercy *et al*., 2014). Also, in the mentioned studies, broth dilution method has been used to evaluate the antifungal effect of garlic aqueous extract, whereas in the present study, garlic aqueous extract has been added to poultry feed. So, it seems that adding the aqueous extract to feed poultry, through raising humidity, provides the ideal conditions for fungal growth.

Conclusion

Based on our results, savory essential oil and garlic powder had significant impact on the reduction of fungal load of poultry feed. On the contrary, the use of these compounds will be associated with fewer possible side effects due to their organic nature. Therefore, it is necessary to survey the possible side effects of these compounds on human and poultry health. If the safety be proved for consumer, these compounds could be used as suitable natural alternatives for controlling microbial load of feed in the poultry industry.

References

1. Adiguzel, A., Ozer, H., Kilic, H., Cetin, B., 2007. Screening of antimicrobial activity of essential oil and methanol extract of Satureja hortensis on foodborne bacteria and fungi. Czech Journal of Food Sciences, 25:81–89.

2. Aly, S.A., Anwer, W., 2009. Effect of Naturally Contaminated Feed with Aflatoxins on Performance of Laying Hens and the Carryover of Aflatoxin B_1 Residues in Table Eggs. Pakistan Journal of Nutrition, 8(2):181-186.

3. Ariyo, A.L., Anthony, M.H., Lami, M.H., 2013. Survey of Mycotoxigenic Fungi in Concentrated Poultry Feed in Niger State, Nigeria. Journal of Food Research, 2(2):128-135.

4. Bakri, I.M., Douglas, C.W., 2005. Inhibitory effect of garlic extract on oral bacteria. Archives of Oral Biology, 50(7):645-651.

5. Berges, R., Siess, M.H., Arnault, I., Auger, J., Kahane, R., Pinnert, M.F., Marie-France Vernevaut, M.F., Bon, A.M., 2004. Comparison of the chemopreventive efficacies of garlic powders with different alliin contents against aflatoxin B_1 carcinogenicity in rats. Carcinogenesis, 25(10):1953-1959.

6. Binder, E.M., Tan, L.M., Chin, L.J., Handle, J., Richard, J., 2007. Worldwide occurrence of mycotoxins in commodities feed and feed ingredients. Animal Feed Science and Technology, 137:265-282.

7. Borhan-Mojabi, K., Sharifi, M., Karagah, T., Karimi, H., 2012. Efficacy of Different Concentrations of Garlic Extract in Reduction of Oral Salivary Microorganisms. Archives of Iranian Medicine, 15(2):99-101.

8. Fani, M.M., Araghizadeh, A.M., 2009. Antifungal activities of fresh garlic extract

on candida albicans ‘(in persian, with English abstract) ‘. Bimonthly Journal of Hormozgan University of Medical Sciences, 13(3):143-148.

9. Hsieh, D.P.H., Atkinson, D.N., 1991. Bisfuranoid mycotoxins: their genotoxicity and carcinogenicity. Advances in Experimental Medicine and Biology, 283:525-532.

10. Irkin, R., Korukluoglu, M., 2007. Control of Aspergillus niger with garlic, onion and leek extracts. African Journal of Biotechnology, 6(4):384-387.

11. Kaushal, K.S., Sinha, S.P., 1993. Mycotoxins. Asean Food Journal, 8:87-93.

12. Khan, A., Hussain, S.M., Khan, M.Z., 2006. Effects of formalin feeding or administering into the crops of White Leghorn cockerels on hematological and biological parameters. Poultry Science, 85:1513-1519.

13. Khan, F.A., Zahoor, M., 2014. Preservation of poultry feed by using different powdered plants. Life Science Journal, 11(1s):9-14.

14. Konjufca, V.H., Pesti, G.M., Bakalli, R.I., 1997. Modulation of Cholesterol Levels in Broiler Meat by Dietary Garlic and Copper. Poultry Science, 76:1264–1271.

15. Krnjaja, V., Stojanovic, L.J., Cmiljanic, R., Trenkovski, S., Tomasevic, D., 2008. The Presence of Potentially Toxigenic Fungi in Poultry Feed. Biotechnology in Animal Husbandry, 24(5-6):87-93.

16. Lemar, K.M., Turner, M.P., Lloyd, D., 2002. Garlic (*Allium sativum*) as an anti-Candida agent: a comparison of the efficacy of fresh garlic and freeze-dried extracts. Journal of Applied Microbiology, 93:398–405.

17. Londhe, V.P., Gavasane, A.T., Nipate, S.S., Bandawane, D.D., Chaudhari, P.D., 2011. Role of garlic (Allium sativum) in various diseases: an overview. Journal of Pharmaceutical Research and Opinion, 1(4):129–134.

18. Low, C.F., Chong, P.P., Yong, P.V.C., Lim, C.S.Y., Ahmad, Z., Othman, F., 2008. Inhibition of hyphae formation and *SIR2* expression in *Candida albicans* treated with fresh *Allium sativum* (garlic) extract. Journal of Applied Microbiology, 105(6):2169–2177.

19. Majd, A., Nejad-sattari, T., Khavarinezhad, R.A., Doosti, B., 2009. Chemical composition of Satureja Khuzestanica Jamzad (Lamiaceae) essential oils produced during ontogenesis and in vitro antimicrobial activity of essential oil ‘(in persian, with English abstract)‘. Journal of Sciences (Islamic Azad University), 18(1):51-60.

20. Mercy, K.A., Ijeoma, I., Emmanuel, K.J., 2014. Anti-dermatophytic Activity of garlic (Allium sativum) extracts on some Dermatophytic fungi. International Letters of Natural Sciences, 19:34-40.

21. Mohammadpour, GH. Majd, A., Najhadsatari, T., Mehrabian, S., Hossinzadehkalagar, A., 2011. Antibacterial and antifungal effects of three genus of thyme plants and two ecotype of ziziphora and satureja bachtiarica essential oils ‘(in persian, with English abstract)‘. Journal of Sciences (Islamic Azad University), 20(1):111-120.

22. Montazeri, S., Jafari, M., Khojasteh, S., 2014. The Effect of Powder and Essential Oil of Savory Medicinal Plant (Satureja hortensis) on Performance and Antioxidant Status of Broiler Chicks under Heat Stress. Iranian Journal of Applied Animal Science, 4(3):573-577.

23. Moreno Romo, M.A., Fernandez, G.S., 1986. Mycoflora of commercial poultry mixed feeds. Poultry Science, 65:284–287.

24. Naoom, R.A.F., 2007. Estimation of aflatoxin residues for some ruminant and poultry (Local and imported) livers in

Mosul. Unpublished Msc thesis. University of Mosul.

25. Olobatoke, R.Y., Mulugeta, S.D., 2011. Effect of dietary garlic powder on layer performance, fecal bacterial load, and egg quality. Poultry Science, 90:665–670.

26. Pennington, L.J., 1986. Mycotoxin: thin layer chromatography and densitometric determination of aflatoxins in mixed feeds containing citrus pulp. Journal of the Association of Official Analytical Chemists, 69:690-696.

27. Qureshi, A.A., Din, Z.Z., Abuirmeileh, N., Burger, W.C., Ahmad, Y., Elson, C.E., 1983. Suppression of Avian Hepatic Lipid Metabolism by Solvent Extracts of Garlic: Impact on Serum Lipids. Journal of Nutrition, 113:1746-1755.

28. Razaghparast, A., Shams Ghahfarokhi, M., Yadgari, M.H., Razaghi Abyaneh, M., 2009. Antifungal effect of allium sativum either individually or in combination with fluconazole, itraconazole and ketoconazole against pathogenic yeasts ‘(in persian, with English abstract), Journal of Gorgan University of Medical Sciences, 11(1):49-56.

29. Razzaghi-Abyaneh, M., Ghahfarokhi, S., Yoshinari, M., Rezaee, T., Jaimand, M.B., Nagasawa, K., Sakuda, S., 2008. Inhibitory effects of Satureja hortensis L. essential oil on growth and aflatoxin production by Aspergillus parasiticus. International Journal of Food Microbiology, 123:228-233.

30. Reeisi, M., Hoseini Aliabad, A., Pashazanusi, M., Rufchaie, A., 2012. The effect of duration and amount use of garlic powder on growth and antibody titers against Newcastle and Gumboro vaccines in broiler chickens ‘(in persian, with English abstract)‘. Journal of Herbal Drugs, 2(4):275-285.

31. Sahin, F., Karaman, I., Gulluce, M., Ogutcu, H., Sengul, M., Adiguzel, A., Ozturk, S., Kotan, R., 2003. Evaluation of antimicrobial activities of Satureja hortensis L. Journal of Ethnopharmacology, 87(1):61-65.

32. Sallam, Kh.I. Ishioroshi, M., Samejima, K., 2004. Antioxidant and antimicrobial effects of garlic in chicken sausage. Lebenson Wiss Technol, 37(8):849–855.

33. Shamim, S., Ahmed, S.W., Azhar, I., 2004. Antifungal activity of Allium, Aloe, and Solanum species. Pharmaceutical Biology, 42(7):491–498.

34. Shams-Ghahfarokhi, M., Shokoohamiri, M.R., Amirrajab, N., Moghadasi, B., Ghajari, A., Zeini, F., Sadeghi, G., Razzaghi-Abyaneh, M., 2006. In vitro antifungal activities of *Allium cepa*, *Allium sativum* and ketoconazole against some pathogenic yeasts and dermatophytes. Fitoterapia, 77:321–323.

35. Shareef, A.M., 2010. Molds and mycotoxins in poultry feeds from farms of potential mycotoxicosis. Iraqi Journal of Veterinary Sciences, 24(1):17-25.

36. Yazdanpanah Goharrizi, L., Sepahdari, A., Sharifpour, E., Sharifrohani, M., Darvishi, D., 2012. Evaluation inhibitory effect of essential oil Savory (Satureja hortensis) in food fish ‘(in persian, with English abstract) ‘. Iranian Scientific Fisheries Journal, 21(3):137-144.

37. Yazdanpanah, L., Mohamadi, N., 2014. Antifungal activity of Satureja hortensis L. essential oil against Alternaria citri. European Journal of Experimental Biology, 4(1):399-403.