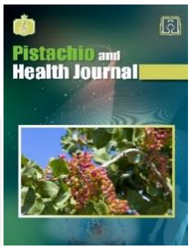
PHJ, Vol. 1, No. 2, Spring 2018, 26-33

Pistachio and Health Journal

Journal homepage: http://phj.rums.ac.ir



REVIEW ARTICLE

Antifungal nanoparticles reduce aflatoxin contamination in pistachio

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Aflatoxins (AFs) are fungal subsidiary products that are predominantly generated by *Aspergillus flavus* and *Aspergillus parasiticus* strains on cereals, nuts, dried fruits and dairy products under warm and humid conditions. Harmful fungi cause spoilage in agricultural crops, and mycotoxins exert harmful effects on humans and livestock. Pistachios (*Pistachio vera* L) are from among the most well-known nut trees extremely susceptible to contamination by aflatoxins and the major contributors to dietary aflatoxins. Aflatoxins, in particular aflatoxin B1 (AFB1), are from among the most toxic natural compounds with carcinogenic effects. Besides, exposure to aflatoxins results in several health-related conditions in humans, including acute and chronic aflatoxicosis, immune suppression, liver cancer/cirrhosis, and stunting. Some traditional and novel control strategies have been proposed for the elimination of AF prevalent in food crops. The application of nanoparticles is a newly advanced method where they are used as antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer agents. Nanoparticles are most widely used for the molecular detection of pathogens at the early stages of the plant growth and for the control of diseases. In this paper, the utilization and effects of nanomaterials on controlling aflatoxigenic fungi and their toxicity on pistachio nuts have been studied.

Keywords: Aflatoxins; *Aspergillus*; antifungal; nanoparticles

1. Introduction

Aflatoxins (AFs) are classified as harmful subsidiary products generated by *Aspergillus* species, specially *Aspergillus flavus* [1] and *Aspergillus parasiticus* [2]. These species have a high outbreak rate that leads to the contamination of a high amount of agricultural products. Considering the broad toxicity impacts of AFs and their role in causing mycotoxicoses in humans and animals, they have a considerable impact on leading to both public health and economic losses. In addition to financial and economic losses to the agricultural industry and animal husbandry industry, food contamination by AF leads to great costs in pharmaceutical and health sectors [3]. Many *Aspergillus* species cause contamination in the nuts and lead to spoilage in immature kernels.

It is demonstrated that over 13 *Aspergillus* species have been separated from pistachio kernels of orchards in Iran and 14 species from orchards in the United States [4]. It has also been reported that 7-45% of the cases of human exposure to AFs have been emanated from pistachios [5]. Aflatoxin-contaminated pistachios lead to a reduction in the level of exports. Due to the high amount of AFs in the pistachios of Iran, the European Union inhibited imports from Iran [5]. Nowadays, regulations on AF have affected the level of crops exports, and Iran is now to challenge the United States to be the best exporter of pistachios. Given the economic losses and public health concerns, the prevention of plant poisoning, including pistachio nuts is required. Although numerous approaches have been proposed against aflatoxigenic fungi and their toxicity, most of them have been criticized due to their low efficiency or contamination concerns. Therefore, new methods such as nanotechnology and nano-phytopathology have been introduced that can be promising in the future.

Nanotechnology is concerned with the synthesis and application of structures with at least one nanoscale

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DOI: 10.22123/PHJ.2018.130469.1007
Received: 12 May 2018
Accepted: 17 June 2018

dimension. Due to their new physical, chemical and biological properties, nanostructures have become of widespread uses in various sciences, with the uses becoming more prevalent day after day [6]. Nanotechnology is also concerned with disciplines such as material engineering, medicine, pharmacy and pharmacy design [7], veterinary medicine, biology, applied physics, semiconductor devices, supramolecular chemistry [8] and also mechanical engineering, electrical engineering, and chemical engineering. From among their uses, one can allude to dental composites, bone cements, implants and medical supplies, weather filters, textiles, cosmetics, packaging containers, household appliances, personal care products, medical devices, and wound dressings [9].

Nanotechnology applications in the field of plant protection include the control of cellular compounds activities without any damage to the cells, the molecular treatment of diseases, rapid disease detection, plant ability boost to absorb required materials, and the provision of productions against toxic products as well as the production of effective fertilizers. Nanotechnology, considered as a powerful technology, has the potential for changing agricultural and food industries across the globe [10]. The advent of nanotechnology and development of nanoscale materials have made it possible for taking advantage of the potential applications of this technology in various agricultural fields [11, 12].

2. Health issues and toxicology of aflatoxin

Aflatoxins are secondary metabolites generated by some molds, especially *A. flavus* species that grow on corns, nuts, seeds, and other common products. Poisoning syndromes resulting from consuming AFs by humans and animals are recognized as aflatoxicosis. The major contaminations have been observed in tree nuts, peanuts and various oilseeds. From among tree nuts, pistachios appear to be specifically susceptible to contamination by AFs. Besides, naturally occurring AF contaminations in pistachios have crucial outcomes for both the global trade and health [5]. B1, B2, G1 and G2 are types of AF that have attracted more attention. After the consumption of contaminated feedstuffs, AFs lead to some negative impacts in animals, ranging from vomiting, a reduction in weight and acute necrosis to the different types of carcinoma, leading to death in the major cases [13]. Even at low levels, AFs predispose animals to infections. Due to the wide-ranging negative impacts, the presence of AFs in foodstuffs is believed to cause harmful effects on health. The strongest and most prevalent carcinogenic materials are naturally generated by *Aspergillus* species [14]. The risk of AF is concerned with their function of prohibiting the production of DNA, RNA, and proteins. Besides, the malfunctions of the enzyme that produces nucleic acids lead to liver necrosis, lymphocyte aggregation [15]. The level, exposure period, age, and nutritional conditions of the animals as well as the synergic impacts of chemical materials to which they are subjected are the factors that determine the harmful effects of AFs. Some subsidiary products generated by *Aspergillus* species are detrimental to animals as well. It is cyclopiazonic acid (CPA) that motivates the necrosis of liver or gastrointestinal tissues as well as necrotic changes to skeletal muscles and kidneys.

3. Economic importance of aflatoxins

Pistachios are considered as the main agricultural export commodity from among other agricultural products in Iran [16]. The global pistachio market is dominated by Iran and the United States (US), and over 70% of the global pistachio exports come from Iran (47%) and the US (25%) [5]. Compared to the 10 ng/g of AF present in the pistachios of the United States, the level of AF in the pistachios of Iran is 54 ng/g [17]. The contamination of nuts with AF is definitely a major issue for a lot of countries that produce pistachios. Due to the high level of AFs in the pistachios of Iran, in 1997, the European Union inhibited the import of pistachios from Iran. If the countries producing pistachios and persons exporting and importing pistachios do not take the high level of AFs in pistachio serious, the condition will deteriorate. Preventing the mold development and AF generation during the pistachio generation and dispensation process can be a good choice. Imposing serious sanitary limitations on orchards, decreasing the growth level of harmful agents in the cultivation phase, and applying a rigorous mechanism for inhibiting contaminations during the harvest and post-harvest phases can lead to favorable outcomes. All individuals playing any role in the pistachio supply chain, including farmers, processors, warehouse keepers, traders, and transporters should be well educated. Food control officials and extension workers should be closely involved in this endeavor. Therefore, considering the importance of pistachios in exports and the value created for the Country by them, developing appropriate supportive policies aimed at strengthening the incentive for farmers to produce and use the new methods of nanotechnology, other methods of preventing mold infections and the AF generation are necessary to prevent a decrease in the pistachio production level [18].

4. *Aspergillus* species and aflatoxin generation

Aflatoxins are extremely toxic secondary metabolites [19] produced by an amount of *Aspergillus* species, including *A. flavus*, and *A. parasiticus* as the major generators; however, some other species like *A. nomius*, *A. pseudotamarii*, *A. parvisclerotigenus*, *A.bombycis*, *A. ochraceoroseus*, *A. rambellii*, and two species with *Aspergillus* anamorphs, i.e. *Emericella astellata*, and *E. venezuelensis* are also AF generators [20]. *Aspergillus* species are saprotrophic and pathogenic molds distributed globally and grow in soil, decaying vegetation, hay, and grains. It is best known for its colonization in cereals, legumes, and tree nuts. Postharvest decay typically increases during harvest, storage, and/or transit processes. *A. flavus* contaminations may occur when hosts are present in the farm (the pre-harvest period), yet they often demonstrate no sign until the post-harvest storage and/or transport periods. Fungal contaminations and the further generation of AF may occur in pistachios in the farm, at the harvest time, during post-harvest activities and the storage period [21]. The level and hazard severity of contaminations depend on the temperature, moisture, soil and storage conditions. In orchards, the nuts most sensitive to contaminations are those weakly supported by the hulls. Early splitting and crust laceration may happen due to late harvest, bird injuries and fractures. Besides, early-split nuts not contaminated in the orchard may become contaminated during the transport and handling periods. The level of AF contamination of tree nuts is not high, but the AF rate is uncertain and a high rate of it can grow in a small amount of nuts. For instance, the findings indicate the contamination rate to be 1 in 28,250 walnuts, 1 in 26,500 almonds, and 1 in 25,000 pistachios. Findings demonstrate that early-split nuts and fractured seedless shells are more prone to the contamination and that contaminations begin to rot them, so defective pistachios can be detected, and by removing them the level of contamination decreases [22].

5. The prevention of pre-harvest mold contaminations and aflatoxin generation

The prevention of mycotoxin contamination is still a critical issue, since farmers are not capable of manipulating the effects of some essential factors on AF generation; these factors include weather conditions, product genotype, the soil type, the lowest and highest daily temperatures and the daily net evaporation rate. Some management practices can be useful as the pre-harvest solution based on Good Agricultural Practices (GAP) like early planting, planting resistant genotypes, applying best farming practices, avoiding drought stresses, adopting proper plant nutrition processes, dominating other plant pathogens, weeds and insect pests as well as following proper harvest practices [23].

5.1. Traditional aflatoxin control methods

5.1.1. Chemical methods

The best antifungal chemical compounds such as propionic acid, phenolic compounds, sodium propionate, benzoic acid, aluminum silicate, citric acid, and azole antifungals are known to affect AF generation [20].

5.1.2. Physical methods

Physical food processing procedures, including dehulling, radiation, extrusion cooking, roasting, baking, frying, and alkaline cooking can effectively remove a major part of AF contamination [20].

5.2. Novel aflatoxin control method

5.2.1. Biological methods

Biocontrol is a promising method for the reduction of both pre/post-harvest AF contamination. The use of non-toxigenic strains, yeasts, bacteria and fungi have proven to decrease AF contamination in Pistachios. Some bacterial isolates such as *Lactobacillus*, *Lactococcus*, *Rhodococcus erythropolis*, *and Bacillu s*sp. have been reported to remove and degrade AFB1. In the same vein, some *Trichoderma harzianum* isolates and the yeast named *Pichia guilliermondii* have been reported to be effective in controlling fungal growth and AF generation [24].

5.2.2. Essential oils

Essential oils (EO_S) as the most essential secondary metabolites extracted from plants play a crucial role in antimicrobial activities. Some essential oils have been classified as “generally recognized as safe” (GRAS) and have a low danger for creating resistance to plant pathogens [25]. The extracts of many plants such as Neem (*Azadirachta indica* Jass.) leafs and the herb *Amorphophallus campanulatus* have been proven to prevent *A. flavus* and *A. parasiticus* growth and AF production [26].

5.2.3. Genetic engineering

The recognition of the genes existing in aflatoxigenic species but not in non-harmful species aimed at altering the genes of toxigenic species to produce in vitro non-harmful species is one of the major challenges and interesting issues in AF_S genetic engineering. Aflatoxigenic *A. flavus* separations indicate four DNA segments, including *aflR*, *nor-1*., *ver-1*., and *omt*-A genes. However, non-aflatoxigenic species show an unstable DNA banding paradigm that lacks one, two, three or four of these genes [27]. Besides, proteomics can function as an instrument to discover plant resistance against fungal contaminations and fungal genetic behaviors [28].

5.3. Nano-phytopathology method

Nanotechnology is a multi-disciplinary science. Nano-phytopathology is a top-notch science that uses nanotechnology for identifying, recognizing and dominating plant diseases and their pathogens at the early stages and screening resistant cultivars using nanobiosensors [29]. Besides, nanoparticles (NPs) like nano-sized silica and silver have been recently utilized as antimicrobial and antifungal agents [30]. Moreover, NPs can be utilized for mycotoxin identification and detoxification, plant resistance boost, plant disease prognosis and the nano-molecular detection of plant pathogens [31].

Table 1. Antifungal effect of nanoparticles on aflatoxigenic fungi

Nanoparticles	Fungus	Major outcome	Ref
Ag NP_S	*A. flavus* var. *columnaris*	Growth inhibitory	[13]
Citrat-coated Ag NP_S	*A. parasiticus*	Reduction of transcriptional aflatoxin gene, two regulators secondary metabolism (laeA and ve A) and Reactive Oxygen Species	[14]
Fe, Cu, and Ag NP_S	*A. flavus and A. parasiticus*	AFB1 adsorption	[40]
ZnO NP_S	*A. flavus*	Growth inhibitory, conidia production and hyphae morphological alteration	[41]
Fullerol C60(OH) NP_S	*A. flavus*	Reduction of mycelial biomass weight and AF production	[42]
C60 NP_S	*A. flavus*	Anti-aflatoxigenic effect	[43]

6. Effects of antifungal nanoparticles

Some studies have reported that NPs could be favorably utilized as substitute antimicrobial agents against diseases created by several drug resistant pathogens. Small-scale NPs with larger volume-to-volume ratios are more effective tools for antimicrobial activities even at very low concentrations. Nanomaterials are also effective and speedy fungicides against a wide range of common fungi, including *Aspergillus* (Table 1), *Candida* [32, 33], and *Saccharomyces* species [34, 35].

The feasibility of silver (Ag) NPs to prevent fungal subsidiary metabolisms at subacute levels creates a new threshold for the detection of customized engineered NPs that can favorably inhibit mycotoxins with the least dangers posed to the health and environment [14]. Because of their strong antimicrobial properties, Ag NPs are used for a variety of applications to control the growth of microorganisms [36, 37]. Nano silver is a potentially antimicrobial agent used against a wide range of gram-negative and gram-positive bacteria, and even against antibiotic-resistant species [38, 39].

Researchers have recently examined the capability of a grape molding fungus, i.e. *Penicillium citrinum* to produce Ag NPs. The ability of bioproduced Ag NPs was checked against aflatoxigenic *A. flavus* var. *columnaris* separated from sorghum grains. An in vitro antifungal examination indicated that bioproduced Ag NPs were capable of preventing the development of aflatoxigenic *A. flavus* var. *columnaris* [13]. In another study, Mitra *et al.* demonstrated that the nonlethal dose of citrate-coated Ag NPs of the size 20 nm could lead to a >2 fold prevention of the bio-production of the carcinogenic mycotoxin and the subsidiary metabolite, i.e. AFB1, in the filamentous fungus and *A. parasiticus* without inhibiting the fungal growth connected with the mycelial absorption of Ag NPs. Exposure to NP led to a remarkable reduction in the transcript rates of five aflatoxin genes and at least two key global controllers of the subsidiary metabolism, i.e. *laeA* and *veA*, with a concomitant decrease in the total reactive oxygen species (ROS). Besides, the elimination of Ag NPs from the growth medium allowed the fungus to fully regain its AF bio-production ability [14].

Asghar *et al.* [40] reported that the antimicrobial activity of biosynthesized iron (Fe), copper (Cu), and Ag NPs against *A. flavus*, *A. parasiticus*, methicillin-, and vancomycin-resistant *Staphylococcus aureus* strains of Ag-NPs showed a better antimicrobial performance compared to Fe NPs and Cu NPs. The adsorbent capability in relation to AFB1 was also assessed in the solution. The adsorption capability of all NPs in relation to AFB1 contaminations was also found in the order of Fe-NPs (131–139 ng/mg) > Cu-NPs (114–118 ng/mg) > Ag-NPs (110–115 ng/mg). Thermodynamic parameters and kinetic studies revealed that the adsorption process is a spontaneous and endothermic process followed the pseudo-second order [40].

The study of the effects of zinc oxide (ZnO) NPs on the harmful species of *Fusarium graminearum*, *Penicillium citrinum,* and *A. flavus* as well as the effects of ZnO NPs on conidia generation, hyphae morphological changes, mortality, and ROS generation showed cell death and ROS generation in the fungi hyphae [41]. Besides, ZnO NPs affect the growth diameter of the fungal colony, the conidia production of all fungi and the hyphae damage.

The impacts of fullerol C60(OH)24 NPs (10, 100, 1000 ng/mL) on mycelial growth, AF generation and oxidative stress regulation were also investigated in an aflatoxigenic strain of *A. flavus* (NRRL 3251) for the incubation period of 168 hours in a liquid culture medium. FNP decreased the mycelial biomass weight partially, but reduced the AF level in the medium remarkably. The activities of the lipid peroxide concentration, superoxide dismutase, catalase, and glutathione peroxidase demonstrated that FNP treatments decreased the oxidative stress within fungal cells hermetically, and in turn suppressed AF generation [42]. In another study, Kovac *et al.* [43] reported that a range of rather low concentrations of nC60 in the environment has the ability to alter AF generation in *A. flavus*. Their findings showed that the NPs of fullerene C60 (nC60) did not display high antifungal properties against *A. flavus*. Besides, nC60 was reported to produce an anti-aflatoxigenic impact at 10-100 ng/mL [43].

Table 2. Antifungal effect of nano-encapsulated antimicrobials on aflatoxigenic fungi

Nanostructures	Antimicrobials	Fungus	Major outcome	Ref.
Chitosan-caffeic acid nanogel	*Cuminum cyminum* essential oils	*A. flavus*	Healing antimicrobial features and persistency of *C. cyminum*	[47]
Chitosan-benzoic-acid nanogel	*Thymus vulgaris* essential oils	*A. flavus*	Increasing the half-time and antifungal features of *Th. vulgaris*	[48]
Chitosan-cinnamic acid nanogel	*Mentha piperita*	*A. flavus*	Improving antimicrobial features of *M. piperita*	[49]
Chitosan NPs	lysozyme	*A. parasiticus*	Spore production and growth inhibitory	[50]

7. Nano-encapsulated antifungals

Nanostructured formulation through mechanisms such as the targeted and controlled release provides the possibility for antimicrobials to release their loads in response to environmental stimuli [44]. In this regard, there is the possibility of using nanotechnology for the development of venues for the retention, encapsulation, and dispersion of materials in a matrix of degradable or inert materials capable of releasing slowly controlled and nutrient-derived elements. Using such nanowires can lead to tremendous achievements such as the high efficiency, environmental compatibility, and targeted delivery [45] of controlled releases [46], and reducing their application may result in pollutions in aquatic and terrestrial environments as well as in foods and agricultural products. Due to the fact that nanostructures have an optimized formulation, it is possible to use them widely in the production of crops (Table 2).

Oils of some plants such as peppermint and *Cuminum cyminum* have significant antifungal features and are extensively evaluated as natural materials for food protection; replacements for harmful fungicides chitosan (CS)–caffeic acid (CA) nanogel were encapsulated and applied to use the antimicrobial feature and persistency of *C. cyminum* oils against *A. flavus*. The efficiency of the encapsulation task was 85% according to the method of optical density spectra of the oil, and the released kinetics examinations showed that 75% of oils that were encapsulated were released in a week. The data indicate that when the oils and environmental factors react, due to the volatility and instability of the oils, the encapsulation of them increases their function remarkably. The minimum preventive levels of free and encapsulated *C. cyminum* oils against *A. flavus* in sealed conditions were 650 and 350 ppm, respectively. Besides, in the non-sealed condition, the performance was better (800 ppm), but the free oils could not inhibit the growth of fungi at the low level (just up to 1000 ppm). These results indicate that by developing nano-carriers, the function of herbal oils can be improved, instead of using lower levels [47]. Khalili *et al.* examined the encapsulated thyme essential oil using chitosan and benzoic acid-made nanogel to improve its antifungal features and half-life. Firstly, the self-formed polymer of chitosan and the nanogel of benzoic acid (CS-BA) were produced, using spectrometric (FTIR) and microscopic procedures (TEM and SEM), and then the size and the shape were determined. Under the sealed condition, the least level of CS-BA encapsulated essential oils with an inhibitory feature was at 300 mg/l, whereas the free oil can just inhibit the development of *A. flavus* at 40 mg/l. Under the non-sealed condition, a higher level of the encapsulated thyme essential oil (500 mg/l) was necessary to fully inhibit the fungi, and the free oils could not reach complete prevention even at the dose 1000 mg/l. It has been proven that encapsulated oils have a remarkable anti-fungal feature in vivo at the levels above the 700 mg/l. In general, free essential oils are volatile and instable, so to increase the half-time and the anti-fungal feature of the thyme essential oil, CD-BA nanogel encapsulation was applied [48].

Beyki *et al*. encapsulated *Mentha piperita* essential oils in nanogel-based chitosan-cinnamic (CS-CI) to improve the antimicrobial feature and stability of the oils against *A. flavus*. The extract of these oils has a significant antifungal feature against *A. flavus,* and encapsulating them can modify their volatility and instability against environmental factors and enhance their function as well. The minimum preventive level of the free and encapsulated *M. piperita* essential oils in the sealed condition was 2100 ppm and 500 ppm, respectively. Besides, the non-sealed condition enhanced the function of encapsulated oils (800 ppm), yet the free oils could not impose full inhibition against *A. flavus* at the levels mentioned (up to 3000 ppm). These results indicated the significant role of CS-CI nanogel as a carrier for essential oils to improve their antimicrobial feature [49].

Hernández-Téllez *et al.* processed chitosan-lysozyme (CS-LZ) NPs via the nano-precipitation procedure, using the chitosan of 153 kDa. TEM and dynamic light scattering (DLS) analyses were conducted to assess the shape, size and dispersion and Z potential. The Coomassie blue assay was performed to assess the efficiency of lysozyme. The antifungal feature of NPs against *A. parasiticus* was assessed by the viability of cells (XTT), decreasing the generation of sugar and the germination and morphometry of spores; the impacts on the integrity of the membrane and wall of the cells were also evaluated. NPs sizes were 13.4 and 11.8 nm for CS-LZ and CS NPs, respectively, and both NPs had a high Z potential level. Besides, the high ratio of lysozyme was rendered to the matrix of CS. According to the biological reactions, CS-LZ NPs decreased the livability of *A. parasiticus* and had a remarkable inhibitory effect on the production of spores (complete inhibition) of in vitro assays at 24 h. CS-LZ; besides, CS NPs influenced the integrity of the membrane and the cell wall of fungal spores compared to the control group. The nanoprecipitation process used to synthesize CS-LZ NPs is a viable and safe method to be used in biological systems, in spite of the low or zero effect on humans and biota. Moreover, because of the possible adverse effects of NPs, their disadvantages and effects on the environment and health should be taken into account [50]. In the same vein, the impact of chitosan, a natural agent for regulating the growth of *A. parasiticus*, was assessed. CS was obtained via chemical (Ch) and biological (Bi) methods from shrimps' heads. Chitosan outputs for Ch and Bi procedures were 5.74 and 6.20%, respectively. The level of the residual protein and the ash was low at 1%, and the molecular weight was low, with the level of deacetylation being 80-82%. Chitosan exerted an inhibitory effect on *A. parasiticus* at the doses of 6.71 and 10.66 g L-1 (CI50) of Ch and Bi, respectively, it exerted an inhibitory effect on the fungal development by 50%. Chitosan (CI50) prevented the radial development and production of spores. Besides, chitosan increased the size of spores and hyphae, shortened the septation procedure and increased the number of the mitotic processes of spores during the production process. However, chitosan reduced the generation of AFB1, but it prompted the whole germination of AFs via *A. parasiticus* compared to the control group [51].

8. Nano-packaging of pistachio

Pistachios are form among the most diverse products, and any adverse internal or external factors can affect their flavor and texture. Packing systems are designed in such a way as to solve such problems, both during and after storage. The high nutritional value of pistachios can be affected by mold infection and other microbiological problems. The inappropriate packing of pistachios also exacerbates their microbial problems. Food packaging is vital because without packaging, the safety and quality of foodstuffs can be compromised [52]. Food packaging is also inclusive, because almost all foods are packed at least in part. The application of nanotechnology to food packaging has increased the shelf-life of foodstuffs [53-55]. The value of the global nano-enabled packaging market for foodstuffs and beverages industry will reach $15.0 billion by 2020.

Some examinations have been performed to remove the barrier against the use of nanotechnology in this filed, particularly in food packaging. Titanium dioxide (TiO2) and low density poly ethylene (LDPE) nanocomposites have been produced as an impediment to oxygen. These nanocomposites have reduced the penetration level of oxygen by 16% compared to pristine LDPE. Hence, these nanocomposites can be used as oxygen barriers and anti-degradation materials in food packaging [56]. The findings of chemical and biological experiments have demonstrated that Ag NPs have had remarkable effects on the lifetime of nuts in the package. The shell lifetime was connected with the level of Ag NPs. Using nano-silver by 3% can produce a highest antimicrobial feature in pistachios. It has been demonstrated that the shell lifetime was lower in the control group, compared to the packaging systems that used Ag NPs. Accordingly, findings show the promising effects of Ag NPs for use in the food packaging [57].

5. Conclusions

From among all mycotoxins synthesized by fungal species, aflatoxins are the strongest hepatotoxic and carcinogenic metabolites, drawing a great deal of attention and being widely studied. In recent years, numerous studies have been conducted on the influence of nanoparticles on mycotoxigenic fungi and mycotoxin production. In order to shed some light on the extent of the correlation between NPs and aflatoxigenic fungi, the effects of NPs on mycelial growth, AF generation and oxidative stress modulation in *A. flavus* and *A. parasiticus* have been examined. The use of NPs and nano-encapsulated antifungals on microbiota, as a great potential for modifying AF production, exhibited both ROS-quenching and ROS-producing properties, while the oxidative stress prompted AF synthesis in the fungi. Moreover, several antifungal NPs are produced by natural resource-based substances that turn them into promising "green" alternatives to traditional fungicidal agents. Despite the progress made so far, health challenges must be addressed to obtain biocompatible and non-toxic NPs with great antifungal properties.

Conflicts of interest

The authors declare no conflicts of interest.

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