Growth and antioxidant responses of strawberry to nickel oxide nanoparticles application

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ABSTRACT

In this study, the potential environmental impact of nickel oxide nanoparticles (NiO-NPs) was investigated on leaf number and length, nitrate content, total phenolic content, chlorophyll a, b and carotenoids contents, lipid peroxidation inhibition, diphenylpicrylhydrazyl radical scavenging activity, nitric oxide scavenging activity, superoxide scavenging activity, and total antioxidant capacity of 'Ventana' strawberry. Thirty days old strawberry plants were treated with four concentrations of NiO NPs (0, 400, 800 and 1000 ppm). Under the highest concentration of NiO, chlorophyll a, nitrate content, total phenolic content, DPPH radical scavenging activity, superoxide and nitric oxide radical scavenging capacities and total antioxidant activity of the extracts were significantly decreased. In lipid peroxidation assay, a non-significant difference was observed between treated and control plants. This study monitored the negative impact of NiO on growth and anitioxidant properties of strawberry plants.

Keywords: Strawberry; NiO-NPs; growth; antioxidant activities; phenolic compounds; radical scavenging.

INTRODUCTION

Strawberry is widely grown worldwide because of its adaptation to various climates and soil conditions (Muradoglu *et al*. 2015). It is consumed in fresh forms and as food-products such as preserves, jams, yogurts and ice creams. Phenolic compounds are secondary plant metabolites that are widespread in the vegetables. They are a large group of biologically active non-nutrients and known to be dietary components in fruits and vegetables with antioxidant activities (Panicol *et al*. 2009). Antioxidants are widely used in food additives and have been studied for pre-emptive role in the development of some diseases such as cancer and heart diseases (Vallee and Ulmer, 1972). They destroy harmful free radicals and prevent cell death (Panicol *et al*. 2009). In strawberries, the phenolic compounds are present as ellagic and *p*-coumaric acid and the flavonoids as quercetin, kaempferol and myricetin (Panicol *et al*. 2009). Strawberry juice contains a high level of antioxidant capacity against free radicals, including superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen (Wang and Jiao, 2000). Moreover, epidemiological studies have shown that eating strawberries is associated with a decrease in blood pressure, inflammation, cancer, and death due to cardiovascular disease (Basu *et al*. 2014).

Nanotechnology is a new emerging and fascinating science with application in industry. The industrial use of nanoparticles is rapidly increasing, and this has given rise to concerns about the environmental fate and potential biological impacts of such engineered particles. The accumulation, persistence and impact of nanoparticles (NPs) on plant metabolism and development depend on the hydrodynamic size, concentration, surface chemistry of NPs, and some other factors (Dietz and Herth, 2011). However, the uptake efficiency and effects of

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Received on 14/9/2015 and Accepted for Publication on 25/4/2016.

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nanoparticles on the growth and metabolic functions vary differently among plants (Dan *et al*.2002, Assuncao *et al*. 2003, Nair *et al*. 2010).

The nickel oxide nanoparticles (NiO NPs) are able to be easily transported into biological systems inducing both cytotoxic and genotoxic effects (Magaye and Zhao, 2012). In plants, accumulation of Ni significantly reduces the yield by decreasing total biomass, numbers of seeds/pod, 100-seed weight and seed yield per plant (Pandey and Sharma, 2002). Plants grown in Ni-contaminated soil show toxicity symptoms including germination retardation (Nedhe, 1990), leaf necrosis and chlorosis (Seregin and Kozhevnikova, 2006; Dan *et al*.2002), inhibition of CO_2 assimilation (Kozlow, 2005), reduction in stomatal conductance (Sheoran *et al*. 1990), growth inhibition and photosynthesis disruption (Gajewska, *et al*. 2006). The NiO NPs vary from spherical to cylindrical, hexagonal, ellipsoidal, and polyhedral in shape. These nanocrystalline particles are agglomerated due to their extremely small dimensions (Hu *et al*.2007). Depending on the particle size, particle shape, and synthesis route, NiO NPs show particular magnetic behaviors such as superparamagnetic, superantiferromagnetic, and ferromagnetic order (Ichiyanagi *et al*.2003). This study was an attempt to understand the effect of NiO NPs on some growth factors and antioxidant activities of 'Ventana' strawberry.

MATERIALS AND METHODS

Thirty days old 'Ventana' strawberry *(Fragaria ananassa* Duch.) plants were obtained from a greenhouse in Shahid Fallah highway of Urmia city, Iran in September 2014. The obtained plants were put into 20 cm diameter plastic pots containing soil and perlite (3:1). Each pot contained two plants and 5 pots were considered for each treatment. Plants were irrigated every 2-3 days and kept at 25 °C under 16 h light/ 8 h dark regime for 2 months under NPs treatment. The experiment was carried out during October and November 2014.

The nickel oxide nanoparticles (NiO-NPs) (Purity: 99%, Average particle size 10-20nm and specific surface area: 50-100 m^2/g) were obtained from Nanosany Co. Mashhad, Iran (Figure 1).The NiO-NPs were applied at concentrations 0, 400, 800 and 1000 ppm immediately after putting the plants into pots. The aerial parts of plants were sprayed from three different directions twice per day at particular times. The leaf and fruit parts of plants were harvested after two months.

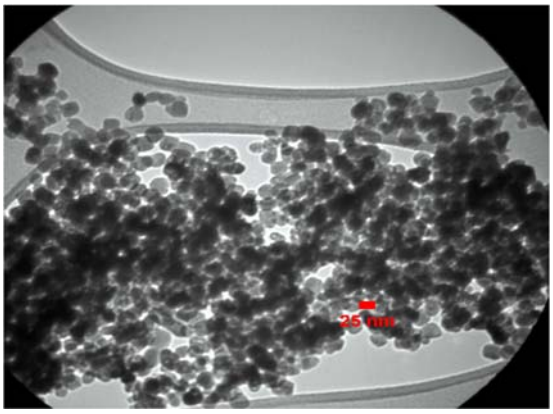

Figure 1. Transmission electron microscope (TEM).

To extract fruits contents, 2 g wet fruits were milled with 25 ml methanol. The extract was placed 3 hours on the shaker and then poured into eppendorf and centrifuged at 3000 rpm for 30 minutes. The supernatant solution of each treatment was distributed between several eppendorfs. The final extracts were kept in the freezer at -80 ºC.

Total phenolic content was measured according to the method outlined by Oki *et al*.2002. Total antioxidant activity and lipid peroxidation inhibitory were determined according to the methods described by Prieto *et al*.1999 and Chawla *et al*. 1976, respectively. Diphenylpicrylhydrazyl (DPPH) radical scavenging activity was measured using the method described by Sanja *et al*. 2009. The chlorophyll a, b and carotenoid contents of leaves were measured using the method described by Lichtenthaler and Wellburn

(1985). For superoxide anion radical assay, the anion radicals were generated by a pyrogallol autoxidation system described by Jing and Zhao (1995). Nitric oxide radical inhibition was estimated using Griess Illosvoy reaction (Garrat, 1964). Leaf nitrate content was measured using method described by Xiong *et al*.2006.

Statistical analysis and experimental design

Data were given as mean ± standard deviation (SD), and significant differences between means were assessed by ANOVA followed by Duncan Multiple Range Test (DMRT), in which $p \leq 0.05$ was considered significant. The SPSS version 13.0 was employed to carry out such analyses.

RESULTS AND DISCUSSION

In this study, the commercially available NiO NPs were suspended in water and used for plant treatment and subsequent toxicity analyses (Figure 1). The current results indicated that strawberry plants exhibited a concentration dependent reduction in leaf number and length. After two months exposure to 800 and 1000 ppm NiO NPs, number of leaves reduced to 14.8 and 14.56, respectively (Table 1). The leaf number in untreated control plants was determined to be 21.3. The NiO NPs at 1000 ppm repressed strawberry leaf length to 3.2cm compared to control plants (4.9 cm) (Table 1).

Nickel has been reported as one of the low consumption and essential nutrients for plants. High concentration of this element causes some changes in the physiological processes causing toxicity, growth reduction (Gajewska, *et al*. 2006), chlorosis and necrosis in plants (Seregin and Kozhevnikova, 2006; Dan *et al*.2002, Ahmad *et al*. 2007). The observed inhibition of leaf number and growth by nickel can be related to inhibition of cell division or prolongation of the cell, or a combination of both (El-Sheekh *et al*. 2003; Vijayarengan and Dhanavel, 2005). The observed negative impact of nickel can also be explained by disturbances in the metabolism of carbon hydrates, proteins and nucleic acids, with an impact on the activity of key enzymes and changing levels of biomolecules such as sugars, amino acids and nucleic acids in plants (Athar and Ahmad, 2002; Pandey and Sharma, 2002).

Nitrate is a critical component of all living systems and nitrate reductase is one of the key enzymes in the assimilation of nitrate. Application of NiO NPs significantly lowered the leaf nitrate content of strawberry plants with the lowest level (11.2 mg/g FW) recorded at 1000 ppm (Table 1). Interestingly, NiO application at 400 ppm induced the nitrate content in strawberry leaves (19.3 mg/g FW). However, this induction did not show any significant change compared to control plants (17.6 mg/g FW).Our data corroborate the earlier study conducted by Chaffei *et al.* (2003) reporting great nitrate content reduction in the roots and leaves of tomato plants treated with cadmium NPs.

Table 1. Effect of NiO-NPs concentration on leaf number, leaf length and nitrate content of 'Ventana' strawberry.

NiO (ppm)	Leaf number	Leaf length (cm)	Nitrate content (mg/g FW)
0	21.3 ± 0.53 ^{a}	4.9 ± 0.07 ^{a}	17.6 ± 0.2 ^{a}
400	17.5 ±0.1 ^{ab}	4.1 ± 0.2 ^{ab}	19.3 ± 0.1 ^{a}
800	14.8 ± 0.3 ^{b}	3.9 ± 0.03 ^{ab}	15.4 ± 0.06 ^{ab}
1000	14.56 ± 0.43 ^{b}	3.2 ± 0.1 ^{b}	11.2 ± 0.1 ^{b}

Statistically significant differences among NiO applications were observed in total phenolic content with the lowest level (0.84 mg/g FW) measured in fruit extracts of plants under 1000 ppm NiO-NPs (Table 2). The total phenolic content in untreated control plants was determined to be 1.42 mg/g FW. The result does not correspond to earlier studies reporting significant increase in phenolic content under Ag-NPs **(**Krishnajar *et al*. 2012**),** heavy metals (Sakihama *et al*. 2002; Schutzendubel and Polle, 2002) and pathogen stress (Lattanzio *et al*. 2006) treatments. Phenolic derivatives have been reported to act as antioxidants reducing ROS. Krishnajar *et al*. 2012 reported elevation level of antioxidant enzymes such as catalase (CAT) and peroxidase (POX) under AgNPs suggesting less ROS generation. Unlike situations mentioned above, in our study, NiO lowered penolic content of strawberry fruits leading to reduction of antioxidant capacity.

Changes in pigment content have direct relationship with physical symptoms of patient plant and its photosynthetic activity (Parekh *et al*. 1990). Hence, chlorophyll contents of plants were measured to determine the effects of NiO NPs environmental stress. The NiO-NPs significantly declined the amount of chlorophyll a of the plants at 1000 ppm (8.01 ± 0.1 mg/g FW) (Table 2). However, application of NiO-NPs at 400 and 800 ppm did not induce any significant effect on chlorophyll a content. Application of NiO-NPs imposed no significant effect on chlorophyll b and carotenoids levels. In the presence of nickel, chlorophyll can be reduced due to increased chlorophyllase enzyme activity or sensitivity of other enzymes in porphyrins synthesis pathway (Gopal, 2002). High concentrations of nickel decrease synthesis of chlorophylls which consequently reduce the rate of photosynthesis (Gajewska *et al*. 2006). Several researchers reported the decrease in chlorophyll content of plants under the influence of heavy metals (Parekh *et al*. 1990; Prasad, 1995; Muradoglu *et al*. 2015). Heavy metals cause severe damage to the photosynthetic apparatus by inducing stomatal closure; chloroplast structure damage, pigments concentration reduction, enzyme disorders and water relations imbalance (Nagajyoti *et al*. 2010).

Malondialdehyde (MDA) is one of the many final products with low molecular weight that is formed from degradation of primary and secondary products of lipid peroxidation (Eriyamremu and Lolodi, 2010). In fact, measuring the level of lipid peroxidation products such as MDA can indicate the amount of damage to the membrane. The lipid peroxidation analysis of fruit extracts revealed a concentration dependent increase in MDA content with the highest level (1.23 μM/g FW) detected at 1000 ppm (Table 2). However, no significant difference was observed between control and plants treated with 400 (1.1 μM/g FW) and 800 ppm (1.19 μM/g FW) NiO-NPs. In accordance with our findings, Zhang *et al.* (2007) detected an increase in MDA content of *B. gymnorrhiza* leaves under exposure to heavy metal stress. Heavy metals enhance reactive oxygen species (ROS) production in a concentration-dependent free radical generation and consequently damage cell membrane (Zhang *et al*. 2007). ROS damage cell membrane, proteins, lipids and DNA resulting in lipid peroxidation (Baccouch and Chaoui, 2001), developmental defects and genetic instability in plant species (Bal and Kasprzak, 2002; Papazoglou *et al.* 2005). Scandalios (2005) reported reduction of antioxidant enzymes level in the presence of high levels of MDA. Although, Ni is not a redox-active metal, and cannot directly generate ROS but interferes indirectly with a number of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), peroxidase (POD), guaiacol peroxidase (GOPX), and ascorbate peroxidase (APX) (Pandey and Sharma, 2002; Gajewska, E. and Sklodowska,2005; Baccouch *et al*. 2001).

Table 2. Effect of NiO-NPs concentration on total phenolic, chlorophyll, carotenoid and malondialdehyde (MDA) contents of 'Ventana' strawberry.

Treatment	Total Phenolic content	(mg/g FW)			MDA
NiO (ppm)	(mg/g FW)	Chl a	Chl b	Carotenoid	(µM/g FW)
0	1.42 ± 0.12 [a]	12.01 ± 0.2 [a]	4.93 ± 0.09 [a]	1.92 ± 0.3 [a]	1.09 ± 0.8 [b]
400	1.14 ± 0.15 [bc]	11.8 ± 0.07 [a]	5.81 ± 0.01 [a]	1.83 ± 0.1 [a]	1.1 ±0.05 [b]
800	1.02 ± 0.2 [bc]	9.83 ± 0.08 [ab]	4.1 ± 0.1 [a]	1.67 ± 0.1 [a]	1.19 ± 0.06 [ab]
1000	0.84 ± 0.21 [c]	8.01 ± 0.1 [b]	4.1 ± 0.1 [a]	1.66 ± 0.4 [a]	1.23 ± 0.1 [a]

To assess the extent of *oxidative* stress in NiO-NPs treated plants, the levels of antioxidant markers such as DPPH, superoxide and nitricoxide radical scavenging activities were determined. The current assay detected 1.69 fold reduction in DPPH radical scavenging activity at 1000 ppm compared to control fruits (Figure 2). Siddiqui *et al*. (2014) reported that heavy metals like Cd, Pb and Cr have a negative effect on DPPH radical scavenging of *Brassica rapa.* In order to protect against the toxic effects of ROS, plant cells employ antioxidant defense systems to provide defense toward any kind of oxidative toxicity at the cellular level (Gill and Tuteja, 2010). Increased level of ROS at higher NiO-NPs concentrations indicates occurrence of oxidative stress due to decreased defense capability (Lee *et al*.2012). Studies show that the toxic effect of nickel on the plants results from their inability to establish a balance between production and elimination of free radicals like superoxide radicals. While excess nickel limit plant growth and cause crop damage over time, the mechanism of nickel action is not yet well known (Frankel, 2005; Scandalios, 2005).

Superoxide and nitricoxide radical scavenging capacities of fruits showed significant decrease with an increase in NiO level, with the lowest activity observed at 1000 ppm treatment. Exposure to this concentration reduced these activities to 1.36 and 1.63 fold lower than control plants. No significant difference was observed between lower application and control for nitricoxide radical scavenging activity (Figure 2). Heavy metals inactivate and down regulate the enzymes of the antioxidative defense system and deplete low molecular weight antioxidants (Aust *et al*. 1985). Due to the reduction of most antioxidant properties of strawberry plants with addition of NiO-NPs, it could be concluded that this NP has toxicity effects on strawberry (Figure 2).

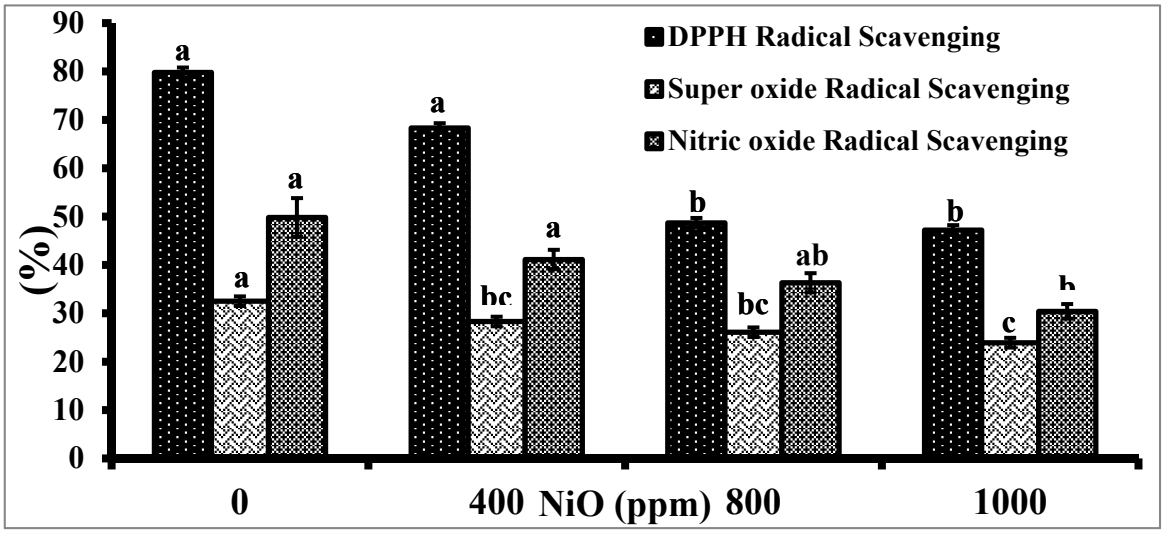

Figure 2. Effect of NiO-NPs concentration on DPPH, superoxide and nitricoxide radicals scavenging activities of 'Ventana' strawberry.

Although, increasing NiO concentration imposed negative impact on DPPH, nitricoxide and superoxide scavenging activities of strawberry fruits, plants did not show any significant difference in total antioxidant capacity among 400, 800 and 1000 ppm applications (Figure 3).

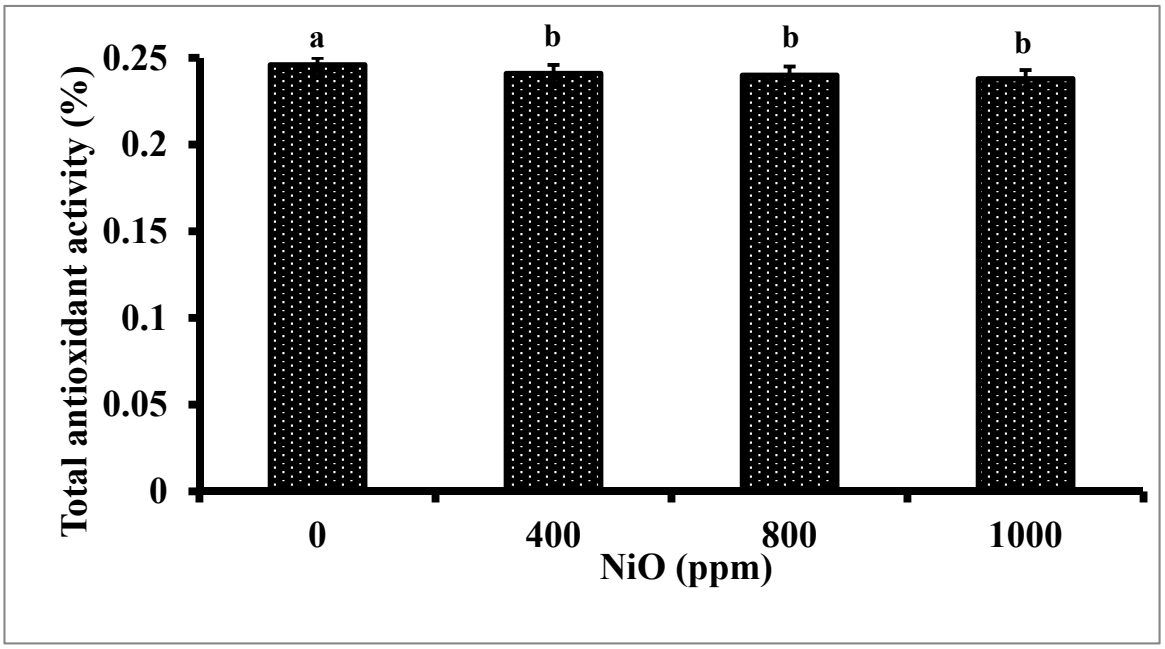

Figure 3. Effect of NiO-NPs concentration on total antioxidant activity of 'Ventana' strawberry.

However, a significant difference was observed between control plants and those treated with higher concentrations. Similar to the current findings, Song *et al*.2013 reported no significant alteration in total antioxidant capacity under different TiO_2 NPs applications in *Brassica campestris* (oilseed rape), *Lactuca sativa* L. (lettuce), and *Phaseolus vulgaris* (kidney bean). The current results indicated strong correlation between phenolic contents and DPPH (R^2=0.89), superoxide (R^2=1) and nitricoxide (R^2=1) radical scavenging activities (Table 3). Treatments with low phenolic content showed low antioxidant activities as well. A positive correlation between antioxidant activity and phenolic content was also reported in *Radix Angelicae* Sinensis (Li *et al*.2009) and Lamiaceae family (Sugihara *et al.* 1999).

Table 3. Correlation coefficients between total phenolic content and total antioxidant activity with DPPH, nitricoxide and superoxide radicals scavenging of 'Ventana' strawberry.

	DPPH radical scavenging	Superoxide radical scavenging	Nitricoxide radical scavenging
Total phenolic content	0.89	1	1
Total antioxidant activity	0.84	0.97	0.96

The current study represents the first report on NiO-NPs effects on strawberry plants at physiological and biochemical levels. It monitored the negative impact of NiO on growth and anitioxidant properties of strawberry plants. Due to the high production and consumption of strawberries in the world (Muradoglu *et al*. 2015) and increasing the production of metal oxide NPs (Stoimenov *et al*. 2002; Niederberger, 2007), the governments and the people should have more and more attention to the problem of heavy metal pollution.

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تقييم تأثير اضافة جسيمات نانو اوكسيد النيكل على نمو ومحتوى مضادات الأكسدة للفراولة

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ملخص

تم دراسة تأثير جزيئات أكسيد النيكل على عدد الأوراق، طول الورقة، محتوى النيتريت ،محتوى الفينول الكلي، تركيز الكلوروفيل أ، ب والكاروتينويدات وتثبيط بيروكسيد الدهون، و DPPH ونشاط كاسح الجذور، ونشاط كاسح أكسيد النيتريك، ونشاط كاسح السوبر أكساید ، ومجموع مضادات الأكسدة في الفراولة صنف فنتانا. تم معاملة نباتات الفراولة (عمرها ثلاثين يوما) بأربعة تراكيز مختلفة من NiO NPs هي 0، 400، 800 و 1000 جزء في المليون. أظهرت النتائج أن التراكيز العالية من NiO قد قامت بتخفيض محتوى الكلوروفيل أ، ومحتوى النيتريت ,محتوى الفينول الكلي، و DPPH ونشاط كاسح الجذور ، ونشاط كاسح أكسيد النيتريك، ونشاط كاسح السوبر أكساید، ومجموع مضادات الأكسدة. بينما لم يكن لمعاملة النباتات ب NiO تأثير معنوي على بيروكسيد الدهون. تبين نتائج هذه الدراسة وجود تأثير سلبي ل NiO على نمو وخصائص مضادات الأكسدة للفراولة.

الكلمات الدالة: الفراولة ،اوكسيد النيكل، النمو، مضادات الأكسدة، المركبات الفينولية، نشاط كاسح الجذور.

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تاريخ استلام البحث 2015/9/14 وتاريخ قبوله 2016/4/25.