Red-pigment production by *Talaromyces atroroseus* TRP–NRC from soybean mill via solid-state fermentation

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Background
Color of food is important to appear its freshness, safety, good processing esthetic, and nutritional values. In the recent years, production of biopigments from natural sources as an important alternative to harmful synthetic dyes is of worldwide interest.

Objective
This study focused on the production of red pigment from local isolated fungus *Talaromyces atroroseus* TRP–NRC by cultivation and optimization of production on soybean mill under solid-state fermentation system.

Materials and methods
A novel local non-mycotoxin-producing fungus *T. atroroseus* TRP–NRC, which is isolated from Egyptian compost and deposited in the gene bank under accession number MW282329, was grown on some agricultural wastes producing industrially red pigment under solid-state fermentation system. About 250-ml conical flasks containing 5 g of substrate, moistened with moisten solution to demand level. The flasks were sterilized, inoculated, and incubated under static condition at 30°C. Studies were conducted for optimization pigment-production conditions, Solid: moisture ratio was tested, 2% of the different carbon sources and equivalent level of nitrogen mg/g substrate were examined. Various pH was adjusted. The effect of incubation temperature was carried out over a range from 20 to 35°C, and the effect of incubation period on pigment production was studied over a range of 5–14 days. Red pigment was extracted from fermented soybean mill with 90% ethanol, solvent was removed using a rotary vacuum evaporator. Estimation of the extracted pigments was done by measuring the absorbance by spectrophotometer OD value at 500 nm.

Results and conclusions
A novel local non-mycotoxin-producing fungus *T. atroroseus* TRP–NRC was grown on some agricultural wastes for producing red pigment (0.129 g/g soybean original substrate) that were achieved under solid-state fermentation system. The highest production yield of red pigment was reached where the initial pH value was 2.5, temperature 25°C after 7-day incubation when the fungus cultivated in fermentation vessel occupied by 2% w/v soybean mill moistened at solid : liquid ratio 1 : 3. Production of red biopigment shows the advantage of mannitol supplementation as carbon source with soybean for increasing pigment by fold. Pigment production was affected positively by addition of organic nitrogen, especially yeast extract higher than addition of inorganic sources and NH_4HSO_4 more suitable than the other tested sources after 7 days. High yield of red pigment (mg/g utilizable soybean) makes the production more economical and encourages to be useful in industrial application.

Keywords:
agricultural wastes, red pigment, solid-state fermentation system, *Talaromyces atroroseus* TRP–NRC

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Introduction

Color of a food material is important to appear its freshness and safety that are also indices of good processing esthetic and nutritional values. In the recent years, production from natural sources is of worldwide interest. These pigments are looked upon for their safe use as a natural food dye in replacement of synthetic ones because of undesirable market. Biocolorants have several applications in food and pharmaceutical sector, as colorants, flavor-enhancing agents, preservatives, vitamins, or nutritional supplement possessing, as well as a lot of beneficial health benefits. Biopigments as organoleptic enhancers in food quality. Biopigments have been applied as nutrient additives in the processing of dairy, fish, meat, juice, and other food products. Biopigments capable of modifying and enhancing many food colors and applied as flavoring agent may display preservative action. A well-textured food, rich in nutrients and flavor, cannot be eaten, unless it has the right color. The demand for production of biopigment is increasing because of the awareness from synthetic colors that caused carcinogenic for man. Therefore, it is essential to explore various natural sources, food-grade natural colorants, and their potentials. Production of colorants by microorganisms plays a significant role as food-coloring agent because of its easy downstreaming process and has many advantages, that is, cheaper production, easier extraction, higher yields through strain improvement, no lack of raw materials, and no seasonal variations. Pigments are used as additives, color intensifiers, and antioxidants [1,2]. Microorganisms are the most powerful creatures in existence and determine the life and death on this planet. Microorganisms are associated with all the foods that we eat and formation of certain food by-products through fermentation, also used as a source of food and food supplements in the form of pigments, amino acids, vitamins, organic acids, and enzymes. In this way, the pigments from microbial sources are a good alternative. Microorganisms are known to produce a variety of pigments, therefore, they are a promising source of food colorants [3]. Many artificial synthetic colorants are widely used in foodstuff, dye, cosmetic, and pharmaceutical manufacturing processes. The synthetic pigments are used to produce harmful effects to human and pollute water and soil [4]. Synthetic colorants are used in food, cloth, cosmetics, painting, plastics, and pharmaceuticals [5]. A series of synthetic dyes are environmentally toxic, potentially adverse allergenic, and intolerance reactions, nonsustainable production processes are strongly correlated with their dependence of nonrenewable resources: coal tar and petroleum [6]. Pigments are from microorganisms of industrial interest because they are more stable and soluble than those from plant or animal sources [7]. Microorganisms can grow rapidly, which can lead to high productivity and can produce a product throughout the year [8]. Several filamentous fungi as potent producers of natural pigments [9,10]. Many authors focused on the strains belonging to the Monascus genus. However, there are other microorganisms, which have the ability to produce pigments in high quantities, such as those belonging to the genus *Paecilomyces* [11], *Talaromyces atroroseus* [12], and Penicillium species [13,14].

This study focused on the production of red pigment from local isolated fungus *T. atroroseus* TRP–NRC, which produced industrially relevant red pigments by cultivation on soybean mill under solid-state fermentation system.

Materials and methods

Microorganism

T. atroroseus TRP–NRC deposit in the gene bank under accession number MW282329 was isolated from Egyptian compost in Microbial Chemistry Laboratory. The fungus is preserved on YPM medium at 4°C and subculture at 3-week duration.

Materials

Agroindustrial wastes were obtained from local market (orange peels, sugarbeet pulp, cornflour, soybean mill, rice flour, fodder yeast, apple pomace, carrot-pulp residue, and pomegranate peels) and industrial food factories in the industrial zone on 6th October City, Giza, Egypt.

Inoculum-seed preparation

The inoculum in the form of spores was prepared by addition of 10 ml of sterilized water to 5-day-old fungal culture slants. The fungal growth was crushed with culture loop, added 0.01% Tween-80 vortexes, and used at 1-ml involved 10^5–10^6 spores to inoculate production flasks, each contained 5 g of substrate.

Experiment

The production was conducted in 250-ml conical flasks containing 5 g of substrate, moistened with moisten solution to demand level. The flasks were sterilized by autoclaving at 121°C for 15 min, cooled to room temperature, then inoculated, and incubated under static conditions at 30°C. Studies were conducted for optimization pigment-production conditions, solid : moisture ratios 1 : 1, 1 : 2, 1 : 3, and 1 : 4 were tested, different carbon sources (2%) were examined, glucose, fructose, lactose, whey, sucrose, galactose, mannitol, xylose, and soluble starch. Different organic nitrogen: peptone, malt extract, and yeast extract combined, as well as inorganic nitrogen at equivalent level: nitrogen mg/g substrate, including urea, ammonium nitrate, ammonium sulfate, potassium nitrate, diammonium phosphate, ammonium oxalate, diammonium hydrogen citrate, and ammonium hydrogen sulfate. The effect of sugars together with nitrogen-source supplementation was carried out by supplementation of mannitol, the more suitable carbon source, and ammonium hydrogen-sulfate nitrogen source together. To check the effect of pH on the production of pigment, the production medium was adjusted to various pH-experiment flasks that were incubated under static conditions for 7 days at 30°C. The effect of incubation temperature was carried out through incubation of fungal culture in temperature over a range from 20 to 35°C. The effect of incubation period on pigment production was studied over a range of 5–14 days.

Pigment extraction

A conical flask that contained fermented matter was mixed with 90% ethanol (adding 5 ml of ethanol per gram of fermented matter on dry-mass basis). The content was mixed on a rotary shaker at 200 rpm for 1 h, allowed to stand for 15 min, and filtered through Whatman No. 1 filter paper. Ethanol extract of unfermented substrate was kept as the blank.

Red-pigment estimation

Estimation of the extracted pigments diluted by ethanol was done by measuring the absorbance by spectrophotometer OD value at 500 nm. The ethanol extracts of unfermented substrates served as blank control.

Red-pigment yield

Red pigment was extracted from fermented soybean mill with 90% ethanol as above-mentioned, and the solvent was removed using a rotary vacuum evaporator. The dry weight obtained was divided by the original substrate.

Results

Screening of some agroindustrial wastes for red pigment production under solid- state fermentation system

Figure 1 illustrates comparative levels of biopigment concentration obtained from cultivating of *T. atroroseus* TRP–NRC on several agroindustrial wastes under solid-state fermentation. The presented results in Fig. 1 revealed that there was conservable variation between agroindustrial wastes. So, soybean mill was the best substrate as the relative pigment yield was 100%, followed by fodder yeast)55%) and then cornflour (42%). The last substrate after that was pomegranate peel that gave the lowest production (7%).

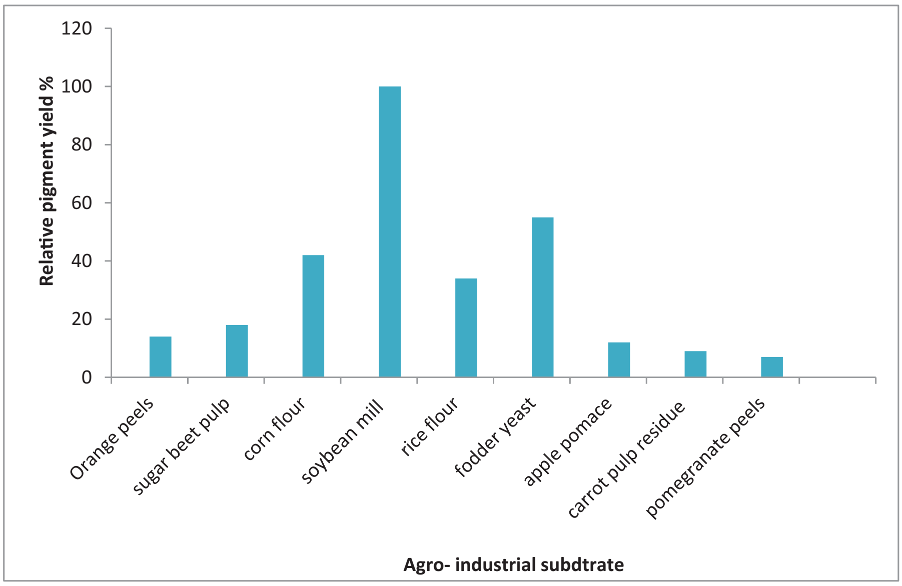
Effect of moisture content

Moisture is the key factor for the successes of solid-state fermentation system for two main factors: the first is to make nutrients involved in the substrate soluble to be metabolizable by microorganisms, the second governs the availability of air in growth medium needed to optimum growth. Figure 2 illustrates that when the solid : liquid ratio was 1 : 3, the extraction of pigment/g substrate was more than another ratio tested.

Effect of substrate to fermentation vessel

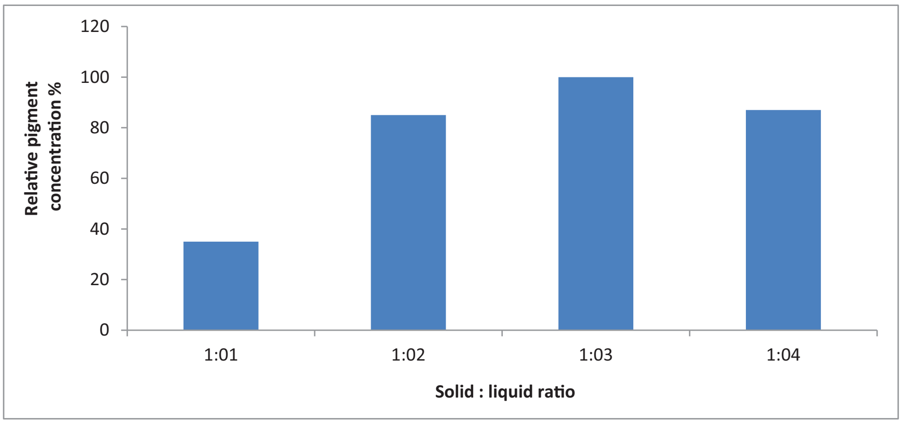
The amount of substrate occupied in the fermentation container governs the oxygen level available for fungus growth. Figure 3 illustrates that the fermentation vessel occupied by 2% w/v was more suitable to give promising red-biopigment production by *T.*

Figure 1



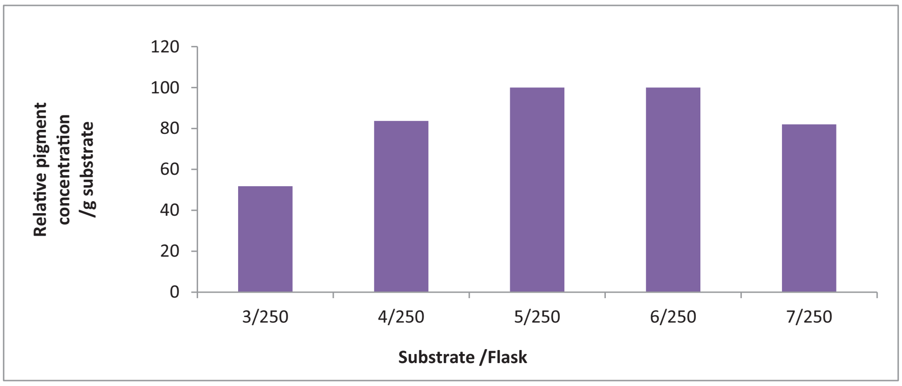
Production of red pigment by *Talaromyces atroroseus* TRP–NRC on several agroindustrial wastes under solid-state fermentation after 7 days at 30°C.

Figure 2



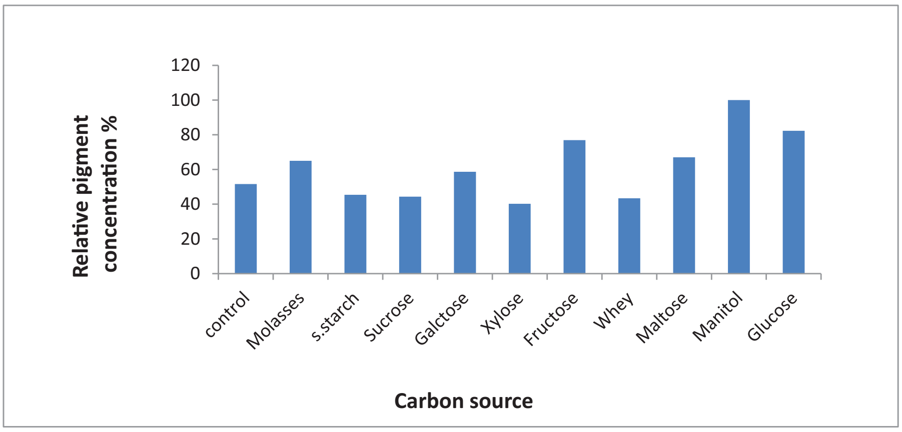
Effect of moisture content on the production of red pigment by *Talaromyces atroroseus* TRP–NRC on soybean mill under solid-state fermentation after 7 days at 30°C.

Figure 3



Effect of substrate to fermentation vessel on the production of red pigment by *Talaromyces atroroseus* TRP–NRC on soybean mill under solid-state fermentation after 7 days at 30°C.

Figure 4



Effect of supplementation of soluble carbon source on the production of red pigment by *Talaromyces atroroseus* TRP–NRC on soybean mill under solid-state fermentation after 7 days at 30°C.

atroroseus TRP–NRC via solid-state fermentation (SSF) of soybean mill 7 days at 30°C.

Effect of soluble carbon-source supplementation

Figure 4 illustrates that between 10 carbon sources were applied to study their effects on increasing the secretion of red pigment by *T. atroroseus* TRP–NRC. Mannitol assists to double-fold for red-pigment concentration production. Glucose and fructose resulted in about 35% increasing in pigment concentration, molasses or maltose increased the pigment production by nearly 25%. A negative effect was seen when soluble starch, xylose, sucrose, and whey were added to fermentation medium.

Effect of inorganic nitrogen source

Figure 5 illustrates that ammonium hydrogen-sulfate supplementation in the growth medium achieved about 25% increasing in red pigment extracted by the fungus *T. atroroseus* TRP–NRC compared with control without any nitrogen addition followed by diammonium hydrogen citrate.

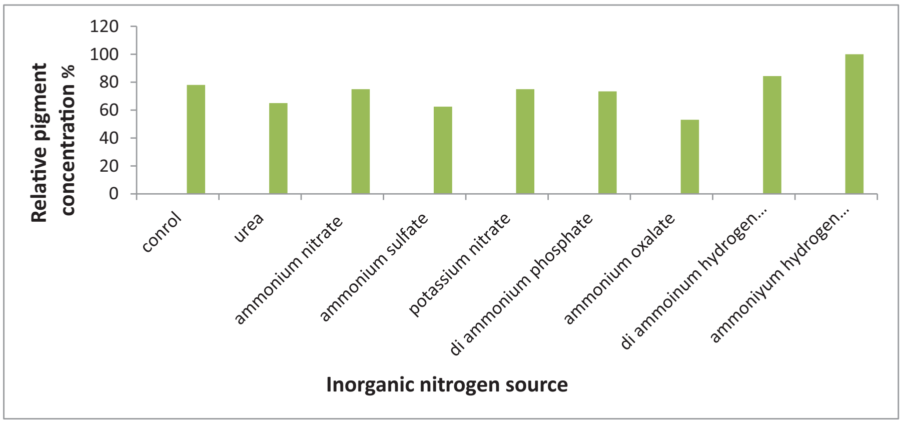
Effect of supplementation of organic nitrogen source

Figure 6 illustrates that however, organic nitrogen source was more favorable than the inorganic one incapable of increasing secretion-pigment production in solid-fermentation medium by *T. atroroseus* TRP–NRC. The supplementation of yeast extract at the level of 6 mg/g substrate achieved about 90% increasing in pigment concentration released in fermentation medium.

Effect of nitrogen and carbon sources together

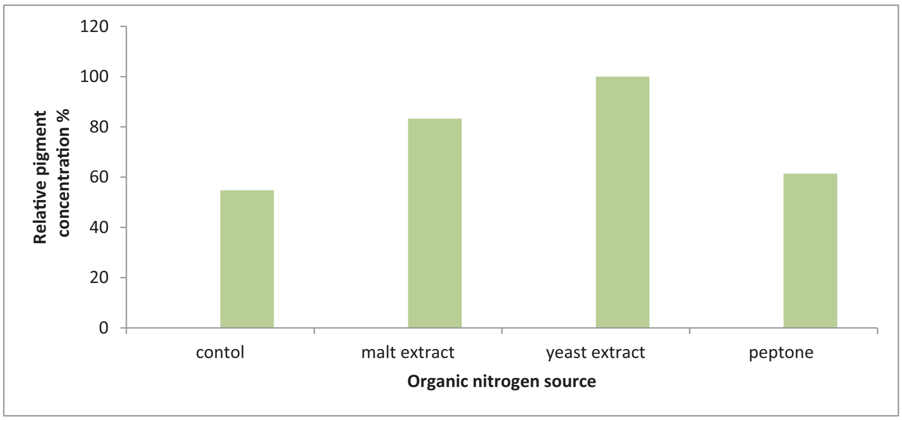
Figure 7 illustrates that pigment-concentration secretion by the fungus is associated strongly by supplementation of inorganic nitrogen, organic nitrogen, or both with carbon source. Addition of

Figure 5



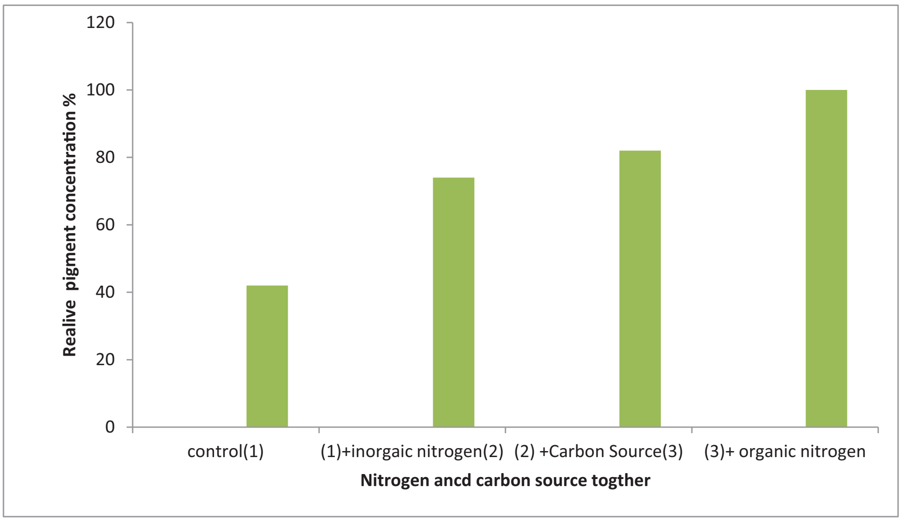
Effect of supplementation of inorganic nitrogen source on the production of red pigment by *Talaromyces atroroseus* TRP–NRC on soybean mill under solid-state fermentation after 7 days at 30°C.

Figure 6



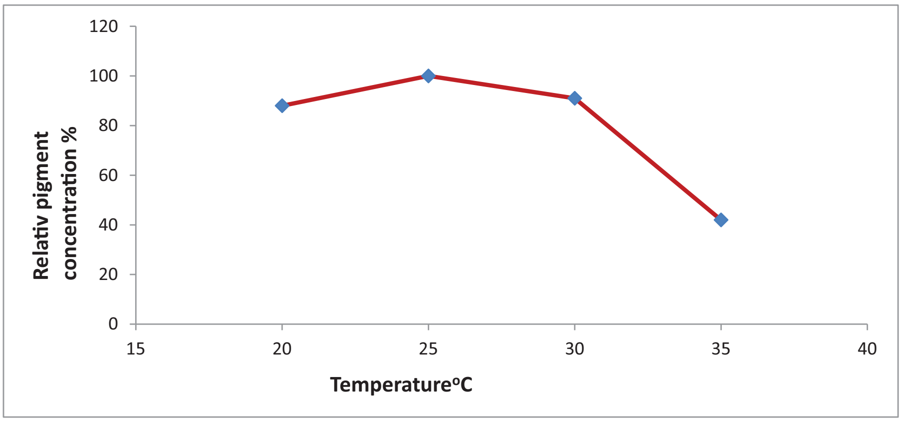
Effect of supplementation of organic nitrogen source on the production of red pigment by *Talaromyces atroroseus* TRP–NRC on soybean mill under solid-state fermentation after 7 days at 30°C.

Figure 7



Effect of nitrogen source with carbon-source supplementation on the production of red pigment by *Talaromyces atroroseus* TRP–NRC on soybean mill under solid-state fermentation after 7 days at 30°C.

Figure 8



Effect of temperature on the production of red pigment by *Talaromyces atroroseus* TRP–NRC on soybean mill under solid-state fermentation after 7 days at 30°C.

inorganic source in the form of ammonium hydrogen sulfate resulted in about 38% increase in the pigment secretion comparable to control without any addition. Mixed carbon source together with previous medium led to doubled increase in pigment secretion by the fungus.

Supplementation of inorganic and organic nitrogen plus carbon source achieved the maximum-secretion pigment in the fermentation medium, which reached about 125% compared with control.

Effect of temperature

Figure 8 illustrates that the maximum production of red biopigment by *T. atroroseus* TRP–NRC tends to be at low temperatures as it was at 25°C. At 35°C, the yield production was decreased dramatically to be about 25% concentration compared with the maximum at 25°C.

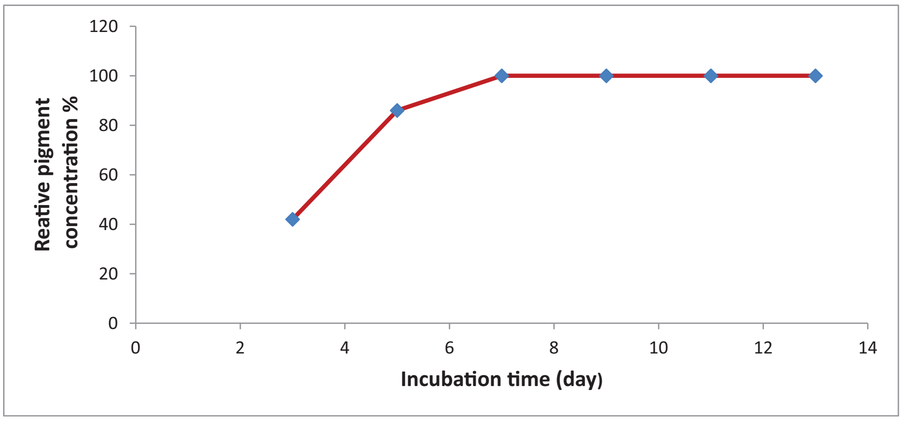
Effect of incubation time

Figure 9 illustrates that red-pigment secretion increased with incubation time and reached its maximum after 7 days, and was then constant.

Discussion

Many different agroindustrial wastes and by-products were tried for red biopigment production by several fungal strains. Jackfruit seed [15] grows in a wide variety of natural substrates. Some natural substrates that have already been tested, besides rice and other cereals, are cassava-starch meal *Monascus purpureus* AHK12 for red-biopigment production by *Monascus*

Figure 9



Effect of incubation time on the production of red pigment by *Talaromyces atroroseus* TRP–NRC on soybean mill under solid-state fermentation at 30°C.

sp. KB10 [16]. Wheat bran, wheat meal, bread meal, corn meal bagasse, coconut residue, soybean meal, and corn were also tested for production of Monascus pigments by a solid–liquid-state culture method [17]. Orange-peel residues were applied for red-pigment production of *M. purpureus* and *Penicillium purpurogenum* [18]. The production of such mycotoxins and drugs limits the use of Monascus for industrial application, but when citrinin has not been found in any *Talaromyces* species, it may be a good alternative fungus for red-pigment product [12]. Systems differed according to microorganisms, substrate type, and nature of substrate, as well as substrate granule size. Lower moisture content led to a large decrease in pigment yield produced by *M. purpureus* Frr 2190 outlived under solid-state fermentation. Researchers tested supplementation of different carbon sources to growth medium to enhance production secretion of pigment produced by many fungi. Molasses were found to enhance pigment production by *M. purpureus* AHK12 cultivated on corn meal or bagasse-coconut residue [19]. Four-percent lactose was found to more suitable addition for increasing pigment production of *M. purpureus* [15]. Xylose 2% (w/v) and glycine 1% (w/v) were used for pigment production of mangrove *Penicillium* species [14]. Glucose was extensively applied for achieving the highest yield of pigments by many fungi, that is, *M. purpureus AHK12* [20,21], *P. purpurogenum* GH2 [13], *Paecilomyces sinclairii* [11], *T. atroroseus* [12], and Penicillium sp. [7]. Our finding reported that mannitol was introduced as a novel sugar-potential supplement for increasing biopigment reproduction by *T. atroroseus* TRP–NRC from soybean mill via SSF. The results obtained can be discussed in the light of the presence of hydrogen ions that makes the medium tend to acidic medium more favorable to red-pigment secretion. Previous studies reported that SSFs of Monascus supplemented with whey or soybean milk as nitrogen sources in the fermentation achieved increasing pigment production [19]. Researchers found that yeast extract stimulates the red-pigment production and gave maximum biomass and pigment yield when cultivated with *M. purpureus* MTCC 410 under solid state [4], peptone for *M. purpureus* [22]. Supplementation of inorganic and organic nitrogen plus carbon source achieved the maximum-secretion pigment in the fermentation medium that reached about 125% compared with control. Previous study shows that cosupplementations of carbon with nitrogen sources in the fermentation medium significantly increased pigment-yield production when compared with the supplementation of each alone [14,23]. The role of temperature and its effect on secondary metabolites, especially pigments, were studied separately for applied fungi. It was found that 25°C was more suitable for producing extracellular red pigment produced by Penicillium strain DLR-7 [14]. The authors stated 35°C [24], others [15] recommended 30°C for the production of Monascus pigments through solid-state fermentation of jackfruit seed [19]. The previous works stated that the high red-pigment production by *M. purpureus* MTCC 410 under solid-state pigment yield was noticed on the 15th day of incubation [4]. It was found that maximum biopigment production was achieved after 8 days by Monascus under solid-state fermentation in a chamber with saturated moist air [25,26]. Researchers cultivated *M. purpureus* on solid-state cultivation and gained the biopigment after 12 days [27], while others harvest good red biopigment from *M. purpureus* FTC5357 after 9 days [28].

Conclusions

This is the first study report about the production of red pigment from new local isolated nonmycotoxin strain *T. atroroseus* TRP–NRC under solid-state fermentation. The technique for the production of red biopigment shows the advantage of mannitol supplementation as carbon source with soybean for increasing pigment by fold from fungus. Pigment yield was affected positively by addition of organic nitrogen, especially yeast extract, higher than addition of inorganic sources, and ammonium hydrogen sulfate was more suitable than the other tested sources after 7 days. High yield of red pigment (mg/g utilizable soybean) makes the production more economical and encourages it to be useful in industrial application.

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Mohamed F and Yomna A.M. Elkhateeb conceptualized, analyzed the data for this work, and necessary inputs were given toward the design of the paper. All authors discussed the methodology and conclusion and contributed to the final paper. Mohamed F sharing in practical section 30%. Yomna A.M. Elkhateeb sharing in practical section 70%. All authors have read the paper and approved it.

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Conflicts of interest

There are no conflicts of interest.

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