Agro-Waste Mediated Silica Nanoparticles and Their Application to Sustainable Agriculture

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Research

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Abstract

Sustainable managing of environment implies the utilization of green approaches in agronomy for growth and crop protection. Since crop production is determined by extensive application of pesticide to resist plant infection and fertilizers to increase fertility of soil, these processes result in ecological unit deterioration as well economic cost. The current research aims at assessment of the potential of nanofertilizers and fungicidal activity of Silica nanoparticles (SiNPs) which have synthesized from agro-waste (sugarcane bagasse and corn cob). SiNPs Nanoparticles display exceptional biological functions and might behave as novel antifungal agents. SiNPs were characterized via scanning electron microscopy (SEM), Fourier transmission infrared spectroscopy (FTIR), X-ray diffraction (XRD) and energy dispersive X-ray (EDX). The plant reaction to SiNPs treatments were observed in relation to germination, growth characteristics, protein, chlorophyll, antioxidant and antifungal activities. Germination rate was enhanced upto 95.5% with SiNPs concentration and growth parameters were increased upto $1000 \mu\text{g L}^{-1}$. The physiological changes showed the increment in protein and chlorophyll content upto 14.8 mg g^{-1} and 4.08 mg g^{-1} , respectively. Other than this, maximum mycelia growth inhibition was reported for *Fusarium oxysporum* and *Aspergillus niger* as 73.42% and 58.92%, respectively. SiNPs positive response on *Eruca sativa* plant might improve its productivity and act as a potential antifungal agent against pathogenic fungi.

1. Introduction

Silica (Si) is found to be the second most abundant element in the Earth's crust and reported as the advantageous element for the agriculture (Tubana et al., 2016). Si exhibits strong positive response towards resistance against biotic and abiotic stress, toxicity of metals as copper, zinc, iron etc. (Mostofa et al., 2021). Previously, no attention was provided to Si treatment for plants because of the high Si content in soil. However, with advancements in agricultural practices, there is requirement for Si incorporation for increasing resistance against stresses and plant growth development (Reynolds et al., 2009). Si is utilized as fertilizers as silicic acid, amorphous silica, and organic complexes. Before Si translocation and accumulation in the shoot parts, it goes through polymerization in root tissues (Debona et al., 2017).

Application of different nanoparticles has resulted in numerous literatures with enhanced growth and development, crop production and resistance against different stresses on plants. Nowadays, silica nanoparticles (SiNPs) are utilized as a major source of Si and has achieved an astonishing position in agricultural sector because of its large surface area and small size, that assures favorable diffusion of SiNPs into root tissues (Hafez et al., 2021). On the basis of their sizes, content and treated plant varieties, SiNPs might behave as positive or negative influence on flora. Nair et al. (2011) displayed that SiNPs (less than 20 nm) reduced seed germination and development of rice seedlings, however SiNPs with size above 20 nm showed favorable results on different plant characteristics. Similarly, studies reported enhanced seed germination in tomato seedlings treated with SiNPs (Siddiqui and Al-Whaibi, 2014).

Valuable role of SiNPs may be possible to work as antifungal agent against pathogenic fungi such as *Fusarium oxysporum* and *Aspergillus niger*. However, literature assessing its antifungal activities against such pathogenic fungi are scarce. More information about the effect of SiNPs against fungal infection is required.

The present study aimed to investigate synthesis, characterization and impacts of SiNPs on physiochemical and biochemical parameters of seedlings of Taramira (*Eruca sativa*). Green method was applied for NPs synthesis using sugarcane bagasse and corn cob as was cost effective, eco-friendly and feasible. SiNPs were characterized through several analytical techniques as scanning electron microscopy (SEM), Fourier transmission infrared spectroscopy (FTIR), X-ray diffraction (XRD) and energy dispersive X-ray (EDX). Effect of synthesized SiNPs was observed on taramira (*Eruca sativa*) seedlings (family: Brassicaceae) via germination percentage, morphological attributes, chlorophyll content, protein and antioxidant enzymes. Other than this, the antifungal potency of prepared SiNPs was tested through disc diffusion assay and broth dilution method. This study will provide a sufficient data to utilize SiNPs in improving the crop productivity.

2. Material And Methods

2.1. Synthesis and characterization of SiNPs

For the SiNPs synthesis, sugarcane bagasse (SBA) and corn cob were collected, cleaned and cut into small pieces. These were sundried and arranged in the muffle furnace for calcination to acquire ashes. Finally, the SiNPs were synthesized through boiling 30g of obtained ash with 1L 1M sodium hydroxide for 1 hr under continuous stirring. The mixture was then filtered, cooled at room temperature and maintained at 7pH for silicon gel production. Synthesized silicon gel was centrifuged and desiccated for 11 hr at 80°C to form xerogel (Fig. 1). Obtained silicon xerogel was rinsed with deionized water to eradicate impurities and stored (Sethy et al., 2019).

Synthesized SiNPs powders were identified and confirmed through different analytical techniques. Morphology of SiNPs were analyzed through SEM (model: MIRA3 TESCAN). Elemental configuration of SiNPs was determined by EDX associated with the FESEM. Other than this, crystalline structure of SiNPs was studied through XRD patterns (Bruker D8 Discover XRD). FTIR spectrum of synthesized NPs was measured through Bruker FTIR spectroscope have in the wave number region of 4,000-400 cm^{-1} (Kumari and Khan, 2017).

2.2. Plant material

Eruca sativa (Taramira) seeds were collected from Sri Karan Narendra Agriculture University Jobner, Rajasthan. Surface sterilization of collected seeds was done via 10% sodium hypo-chlorite (NaClO) solution for 10 min and germinated into petri plates (50 seeds/plate) placing a double layer of filter paper. After 5 days, seedlings were transferred into the hydroponic solution pots.

2.3. Hydroponic cultivation

Hoagland solution was prepared through the combination of following macronutrients (g L^{-1}) as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, KNO_3 , NH_4NO_3 , KH_2PO_4 , H_3BO_4 , $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and micronutrients as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, KI , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, Na_2EDTA and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$. SiNPs. Treatment of SiNPs were given with concentration range $100\text{--}1000\mu\text{g L}^{-1}$ (Singh et al., 2015). Entire study was conducted in plant growth chamber under controlled environment (photoperiod of 12 hr at room temperature) and arranged as completely randomized block designs in replicates.

2.4. Toxicity assessment

Four different concentrations as 100, 250, 500 and $1000\mu\text{g L}^{-1}$ of SiNP was prepared. Surface sterilized 50 seeds were placed in their respective petridishes, thereafter suspension of SiNP each concentration was supplemented to each petridish of respective treatment. For the germination, petridishes were kept in dark with adjusted conditions. Firstly, germination rate was calculated based on the seeds germinated and seeds with root tip 1 mm and upper were considered as germinated. Root-shoot length (mm) and biochemical experiments were conducted at time interval of 3, 6 and 9 days after germination of seeds.

Polyphenol analysis

Bray and Thorpe (1954) method was utilized for polyphenol content analysis with 0.1 g ground leaves in 75% methanol. Further, absorbance was recorded at 725 nm using spectrophotometer after addition of 25% sodium carbonate.

Determination of Chlorophyll content

Chlorophyll content of plant was determined through method illustrated by Li et al. (2016). Seedling at 3, 6 and 9 days' time interval were homogenized with acetone and incubated overnight. Chlorophyll content was determined as follows:

$$\text{Chl a (mg/l)} = 12.72 (A_{663}) - 2.59 (A_{645}) \text{ (a)}$$

$$\text{Chl b (mg/l)} = 22.88 (A_{645}) - 4.67 (A_{663}) \text{ (b)}$$

$$\text{Total Chlorophyll content (mg/l)} = \text{Chl a} + \text{Chl b} \text{ (c)}$$

Total Protein

Bradford (1976) method was utilized for calculation of total protein at different time intervals. For this, 100 μl of enzyme extract was mixed with 1 ml of Bradford solution and absorption was recorded in 595 nm wavelength.

Antioxidant activity

Antioxidant potential of the plant was detected through monitoring the activities of different enzymes as SOD and PAL. For antioxidants analysis, leave samples (2.0 g) from non-treated and treated seedlings

were rinsed and extracted in 10 ml extraction buffer. The obtained crude extract was filtered through Whatman filter paper and centrifuged at 13,000 rpm for 30 min. at 4°C.

Peroxidase (Pox) activity was analyzed through the method given by (Güneş et al. 2019). For this, 3ml of solution containing 0.5 ml guaiacol solution in 0.1 ml sodium phosphate buffer of pH 7.0, 0.3ml hydrogen peroxide and 0.1 ml of enzyme extract were mixed. Pox activity were documented using spectrophotometer at absorbance 436nm, after every 30sec. upto 3 min. Activity was calculated utilizing the extinction coefficient of guaiacol at 436nm.

Superoxide dismutase (SOD) activity was evaluated using nitrobluezolium tetrazolium (NBT) in the presence of riboflavin (Güneş et al., 2019). The reaction mixture was prepared through mixing of 50µl enzyme extract, adding 1 ml NBT (50µM), 500 µl methionine (13mM), 1 ml riboflavin (1.3 µM), 950 µl(50mM) phosphate buffer and 500 µl EDTA (75mM).

2.5 Microscopic analysis

SEM (model: MIRA3 TESCAN) associated with EDX was utilized for the confirmation of SiNPs accumulation in root, shoot and leaf tissues. For this, the plant tissues were fixed with 0.5 M phosphate buffer containing 2.5% of glutaraldehyde, left overnight and dehydrated with alcohol concentration series (Kumari and Khan, 2018). Initially, plant tissues were analyzed through light microscopy and further examined through FESEM. Other than this, EDX analysis confirmed the presence of SiNPs in root, shoot and leaf tissues.

2.6 Media for test fungus and culture conditions

Strains of *Fusarium oxysporum* and *Aspergillus niger* were procured from MTCC, Chandigarh. The fungus was cultured on potato dextrose agar (PDA) at 27°C temperature. Further, the spores of both fungus strains were collected from PDA culture plates after 7 days incubation (Liu et al., 2002). Both the fungal strain spore suspensions were stored in 20% glycerol at -40°C for further application.

Disc diffusion assay

Both strains fungal spores were inoculated on agar media and 2 mm filter discs were placed. Further, the synthesized SiNPs suspension was poured onto placed filter discs and the zone of inhibition was recorded in mm after 24 hr (Balouiri et al., 2016).

Minimal inhibitory concentration (MIC)

MIC was determined as the minimum concentration of biogenic SiNPs that can inhibit the fungal growth. MIC values for both strains were correlated with the synthetic fungicides and control samples (without SiNPs). All the MIC experiments were performed in triplicates. After 48 hr of incubation, 5–10µl broth from each vials were poured onto freshly autoclaved agar media (Basha and Ulaganathan, 2002).

Statistical analysis

Results were calculated through variance (ANOVA) test. Individual bar in the data denotes mean $\pm$ standard deviation of three replicates followed by ‘*’ means were significantly different ($p \leq 0.05$) by tukey’s test.

3. Results And Discussion

3.1 Characterization of synthesized SiNPs

SEM micrograph confirmed the irregular morphology of SiNPs with an average size range from 20 to 40nm (Fig. 2a).Maximum nanoparticles were reported settled in nano-agglomerated state. EDX spectrum depicted 52.84% of silica (Si) along with 47.16% of oxygen (O) (Fig. 2b).SiNPs were also subjected to XRD analysis for the confirming the crystalline structure. Major peaks of synthesized SiNPssamples were measured at $2\theta = 36.01^\circ$, 32.11° , 46.10° and 57.13° (Fig. 2c). The diffraction peaks reported were similar to the standard patterns of JCPDS file no: 01-0787, which confirmed the crystallographic structure of SiNPs. Average size of SiNPswas calculated through Debye-Scherrer equation (Yew et al., 2016).

Debye-Scherrer equation is shown as:

$$D = k\lambda / \beta \cos \theta$$

The average crystallite size of SiNPs calculated was 17.23 nm. XRD pattern confirmed the high crystalline purity of SiNPs assimilar results were reported(Rangaraj et al., 2012; Chanadee and Chaiyarat, 2016).

FTIR spectrum analyzed the chemical nature and functional groups of the synthesizedSiNPs.The peak observed at 1073 cm^{-1} corresponds to the asymmetric stretching vibration and shear bands of Si-O-Si bonds (Palanivelu et al., 2014). The peak band observed at 800 cm^{-1} corresponds to the symmetric stretching vibration of Si-O bonds (Palanivelu et al., 2014). The peaks observed between 1090 and 799 cm^{-1} as present at 1111.39 cm^{-1} revealed the vibrational mods of silica gel network. Other than this, band at 1645 cm^{-1} was reported corresponded to the adsorption of silanol OH groups (Fig. 2d) (Palanivelu et al., 2014). The average diameter and zeta potential of the SiNPs reported through zeta-analyzer was 38.28nm and -37.1 (Fig. 2e).

3.2 Effect of SiNPs on *Eruca sativa*

The outcomes from effect of SiNPs indicated positive effects on the majority of the assessed morphological parameters in *Eruca* seedlings (Fig. 3a-d). For the observation, seeds germination was measured through observing the radical presence. Seedlings not treated with SiNPs were indicated as control. All seedlings applied with SiNPs clearly showed enhanced germination, shoot and root lengths.Maximum germination percentage of seeds was observed as 92% after 48 hr of SiNPs treatment (Fig. 4a). Highest shoot length was reported as 6.3 cm at $1000 \mu\text{g L}^{-1}$ SiNPs treatment after 9 days interval; while minimum shoot length was observed as 5.5 cm in control ones (Fig. 3c). At the same time,

maximum root length was measured as 6.6 cm at 1000 $\mu\text{g L}^{-1}$ SiNPs treatment while minimum root length as 6.3 cm in control seedlings (Fig. 3d). Overall, results showed that root and shoot length were reported slightly increased at lower concentrations as 250 $\mu\text{g L}^{-1}$ and 500 $\mu\text{g L}^{-1}$ and highest was reported at higher concentration (1000 $\mu\text{g L}^{-1}$).

Similar results were reported for treatment of SiNPs, that increased the root-shoot length and development of cucumber seedlings, however after certain concentration slight reduction was observed. Maximum root-shoot length was reported at 200 mg L^{-1} SiNPs concentration as 6.51 and 5.10 cm (Alsaeedi et al., 2019). Above observation agree with the studies of Nair et al. (2011). This study reported increased germination of rice seedlings with the treatment of FITC-labelled SiNPs. Therefore, on the basis of outcomes after SiNPs treatment, we may perhaps simply picture their straight and indirect participation in the plant development (Fig. 3a-b) through increment in seed germination properties.

Polyphenol content

Phenols are major defensive compounds which defend plants against numerous stressors as phenols plays a significant part in absorbing and deactivating free radicals and decomposing peroxides (Shah et al., 2010). Influence of SiNPs on the polyphenol content of treated *Eruca* seedlings is shown (Fig. 4b). Polyphenol content was linearly accelerated with the treatment of different concentration of SiNPs. Highest polyphenol concentration was observed at 1000 $\mu\text{g L}^{-1}$ SiNPs treatment and minimum was reported at 100 $\mu\text{g L}^{-1}$ SiNPs treatment at 9 days interval. Similar results were observed as SiNPs applications improved the buildup of phenolic compounds in leaf epidermis compared to untreated. The mechanism of SiNPs-induced phenols may be because of the insoluble SiNPs, which is accumulated in epidermis, that induce the enrichment of constitutional phenols in epidermal cells by their super high adsorption surface (Li et al., 2004).

Determination of Chlorophyll and protein content

Leave samples were collected from treated and control pots that showed the chlorophyll a and b contents were significantly increased with increasing SiNPs concentrations and time interval (Fig. 5a). Chlorophyll a, b and total content was found maximum at 1000 $\mu\text{g L}^{-1}$ SiNPs concentration and lowest at 100 $\mu\text{g L}^{-1}$ SiNPs. Similar results were obtained in study conducted by El-Serafy, 2019. These results were also favored by the research done by Sun et al. 2016 where mesoporous SiNPs increased the chlorophyll a, b and total concentration (Sun et al., 2016).

Outcomes depicted increment in the total protein content at 3, 6 and 9 days' time interval for all SiNPs treatment (100, 250, 500 and 1000 $\mu\text{g L}^{-1}$). However, the reported total protein content at 100 $\mu\text{g L}^{-1}$ treatment showed no significant difference as compare to control pots (Fig. 5b). Maximum protein content was observed at 500 $\mu\text{g L}^{-1}$ SiNPs treatment as compared to 100, 250 and 1000 $\mu\text{g L}^{-1}$. At highest SiNPs concentration as 1000 $\mu\text{g L}^{-1}$, protein content was decreased which showed the toxic

effect of SiNPs above certain concentration. Similar results of protein content increment upto certain level were reported for study conducted by Sun et al. 2016.

Impact on oxidative stress

Antioxidant enzymes and metabolites strongly control the ROS formation and their lethal impacts under oxidative stress conditions. With the increasing concentrations of SiNPs, the antioxidant potential in terms of the activity of the antioxidant enzymes elevated in the seedlings. Opposing to other previous literatures, the action of both antioxidant enzymes, i.e., POD and SOD reduced with elevating SiNPs treatments even with the similar treatment route. Spectrophotometric observation depicted that peroxidase activity (POD) decreased with increasing SiNPs treatments (Fig. 6a). POD activity decreased at all SiNPs treatments as $100\text{--}1000\text{ }\mu\text{g L}^{-1}$. The superoxide dismutase (SOD) and polyphenol oxidase activity also decreased with increasing SiNPs as $100\text{--}1000\text{ }\mu\text{g L}^{-1}$ (Fig. 6b). This decrement can be because of the healthier growth medium and nourishment provided through SiNPs. In similar study, cucumber seedlings depicted decreased amount of ROX species (i.e., H_2O_2) with the treatment of SiNPs (Alsaedi et al., 2019). Similar results were reported as a linear decrease in the activity of POD upon increasing the dose of applied SiNPs. The highest POD activity was reported at 200 mg L^{-1} SiNPs through the soil treatment (S200), whereas the lowermost POD activity was observed for the concentration of F200S600. Other than this, foliar treatment of 200 mg L^{-1} showed minimum POD activity as compared to the soil treatment of the similar concentration (Attia and Elhawat, 2021).

3.3 Microscopic studies

SEM micrograph exhibited the uptake and accumulation of SiNPs in leaf, shoot and root tissues of treated seedlings (Fig. 7) SEM images of transverse sections (T.S.) of shoot and root tissues at $1000\text{ }\mu\text{g L}^{-1}$ SiNPs treatment at 9 days interval showed the NPs presence (Fig. 7c and e). SEM images of leaf tissues also depicted the SiNPs presence (Fig. 7a). For further confirmation, EDX analysis confirmed the existence of Si in leaf, shoot and root tissues through the presence of Si peaks (Fig. 7b, d, f).

3.4 Antifungal activity

The antifungal strength of SiNPs was determined against *Fusarium oxysporum* and *Aspergillus niger* mycelia on nutrient agar plates through disc diffusion assay. A control plate was independently maintained for both fungal strains with absent SiNPs. The zone of fungal inhibition by SiNPs synthesized from agro-waste (corn-con & sugarcane bagasse) was observed for both *Fusarium oxysporum* and *Aspergillus niger* and no zone of inhibition was reported in control plates. Maximum % inhibition reported in *Fusarium oxysporum* and *Aspergillus niger* was 73.42 ± 1.14 and 58.92 ± 3.49 at $1000\text{ }\mu\text{g}$ SiNPs concentration, respectively (Table 1). Minimum inhibitory concentration reported for *Fusarium oxysporum* and *Aspergillus niger* were 3.1 and $6.3\text{ }\mu\text{g/ml}$. Similarly, increased antifungal efficiency of mesoporous SiNPs against *Alternaria solani* was reported for the tomato plants. This laboratory synthesized mesoporous SiNPs displayed noticeable increment in tomato plant development as compared to untreated plants (Derbalah et al., 2018).

Similar study was conducted for pathogenic fungi as *Fusarium oxysporum* and *Fusarium oxysporum* with SiO₂NPs on potato dextrose agar (PDA) plates. Highest inhibition efficiency almost 95% for both fungi was reported in the study (Akpinar et al., 2017).

Table 1
Percentage (%) of Growth inhibition of *Fusarium oxysporum* and *Aspergillus niger* by SiNP.

Fungus	SiNPs content					
	1000 µg	500 µg	250 µg	100 µg	Standard	Control
<i>Fusarium oxysporum</i>	73.42 ± 1.14	64.28 ± 2.30	61.30 ± 1.69	53.1 ± 1.52	M (97.67 ± 0.07)	0.00 ± 0.00
<i>Aspergillus niger</i>	58.92 ± 3.49	51.1 ± 2.79	43.7 ± 1.90	41.5 ± 1.58	M(100)	0.00 ± 0.00
Each value represented in table are means ± SD (N = 3), 0.00: Indicates no inhibition, (M)- Manocozeb						

Conclusions

The present study reveals an effective and profitable green process for the preparation of SiNPs from SBA and corn cob. The application of SiNPs to the *Eruca* seedlings enhanced not only the plant biometrics and plant physiology but also acted as antifungal agent. SiNPs treatments were applied in the range of 100, 250, 500 and 1000µg L⁻¹ at 3, 6 and 9 days interval. These possible effects of SiNPs on plant growth and enhancement in the photosynthetic pigments, protein content, polyphenols and decrement in antioxidant capacity. However, both SOD and POD antioxidant activities were decreased with increasing SiNPs concentration. The study indicates that SiNPs at optimal concentrations as 1000 µg can behave as antifungal agent. SiNPs showed maximum inhibition percentage as 73.42 and 58.92 for both *Fusarium oxysporum* and *Aspergillus niger*.

Abbreviations

SiNPs- Silica nanoaprticles

SEM- Scanning electron microscope

FTIR- Fourier transmission infrared spectroscopy

XRD- X-ray diffraction

EDX- energy dispersive X-ray

SBA- Sugarcane bagasse

MIC- Minimum inhibitory concentration

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

The authors have consent for publication.

Availability of data

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

Funding

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Authors' contribution

JM and PG conceived the present idea of writing the research and designed the content. JM encouraged to investigate and supervised the findings of this work. PG developed the theory and performed the experiments. JM and PG contributed to the analysis of the results and writing of the manuscript.

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Figures

Figure 1

Schematic representation of SiNPs synthesized from agro-waste (Sugarcane bagasse and corn cob).

Figure 3

Effect of SiNPs on *Eruca sativa* plant (a and c) shoot length and (b and d) root length at 9 days interval at 100 to 1000 $\mu\text{g L}^{-1}$ concentration.

Figure 4

Effect of different concentrations of SiNPs on *Eruca sativa* a) germination percentage at 48h with concentration 100, 250, 500 and 1000 $\mu\text{g L}^{-1}$ of SiNPs and b) Polyphenol content with same treatment.

Figure 5

a) Chlorophyll a (Chl a),Chlorophyll b(Chl b) and total chlorophyll content and b) protein content in fresh leaves of *Eruca sativa* with 100 $\mu\text{g L}^{-1}$, 250 $\mu\text{g L}^{-1}$, 500 $\mu\text{g L}^{-1}$ and 1000 $\mu\text{g L}^{-1}$ of SiNPs after 9 days hydroponic cultivation.

Figure 6

Antioxidant activity (a) POD and (b) SOD of *Eruca sativa* with 100, 250, 500 and 1000 $\mu\text{g L}^{-1}$ of SiNPs after 9 days.

Figure 7

(a) and (b) show the FESEM and EDX spectrum of leaf tissues, (b) and (c) shoot tissue and (e) and (f) root tissues of Eruca seedlings at 1000 $\mu\text{g L}^{-1}$ SiNPs treatment after 9 days.

Supplementary Files

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