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<https://esciencepress.net/journals/phytopath>**FORMULATION OF NANO-FERTILIZER AND PHYTOCHEMICAL SCREENING OF ORANGE PEEL UTILIZED TO ENHANCE THE GROWTH OF *VIGNA RADIATA***^aSaba Iqbal, ^aGulnaz Parveen, ^bAmbreen Ayub, ^cSalma Gul, ^dTahira Batool, ^eNain Tara, ^fAmtul Sami, ^gAtiya Hussain Khowaja^a Department of Botany, Women University Swabi, Pakistan.^b Department of Physics, Women University Mardan, Pakistan.^c Department of Chemistry, Women University Swabi, Pakistan.^d Department of biotechnology, Women University of Azad Jammu Kashmir Bagh, Pakistan.^e Pathology/Microbiology Section, Bahria University of Health Sciences (BUHSC) Medical Laboratory Technology, Pakistan.^f Department of Health Biotechnology, Women University Swabi, Pakistan.^g Agha Khan Medical University and Hospital, Karachi, Pakistan.**ARTICLE INFO****Article History**

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ABSTRACT

Nanotechnology has developed into one of the most valued field of sciences in the current century owing to introduction of revolutionary changes in several disciplines of sciences. A key product of nanotechnology i.e. nanoparticles (NPs) are abundantly used in modern research due to their novel applications. Orange peel contains vital nutrients that can be recycled into valuable material used for different purposes. In this study, we synthesized nano fertilizer from orange peel and tested its potential for promoting the growth of *Vigna radiata* and reducing the population of root rot pathogens. Nano-fertilizer extract was subjected to physical and chemical analyses for characterization. Transmission electron microscopy indicated spherical nanoparticles with sizes ranging between 18.22nm and 61.05nm. While the X-Ray diffraction analysis the peaks were observed at 2θ value of 19.830, 20.550, 21.240, 24.440, 27.330 and 29.770 which represented the similarity with urea slandered peak. The synthesized nano-fertilizers contained phenols, steroids, triterpenes and Xanthoproteins. In a screen house experiment, the nano fertilizer extract was administered to the seeds of *Vigna radiata*. The research demonstrated that increasing the dose of orange peel extract enhanced germination percentage by positively influencing plant growth characteristics. Meanwhile, a high concentration of orange peel amendment successfully reduced the colonization and infection percentage of root rotting pathogens when compared to the positive and negative controls.

Corresponding Author: Gulnaz Parveen

Email: gulnaz.parveen@wus.edu.pk

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INTRODUCTION

Orange (*Citrus sinensis*) is a delicious, juicy fruit from the Rutaceae family. It is one of the world's most widely grown fruit crops, having significant commercial applications in food, pharmaceutical preparations, perfumes, and cosmetics due to the presence of essential

oils in its peel (flavedo) (Farahmandfar *et al.*, 2020). Antioxidant, antibacterial, insecticidal, antimycotic, and cytotoxic activity of essential oils are among the biological properties identified for this *C. sinensis* peel (Oyedeji *et al.*, 2020). The family Rutaceae has four genera t of which one of the most important genera is

Citrus. Orange is commonly found in tropical and subtropical regions of the world. It has a high nutritional value as 100 grams of orange contain 46 kilocalories, 86% percent water, and 0.9% protein.

Orange peel contains cellulose, pectin, hemicellulose, carotenoid, coumarins, essential volatile oils, lignin, phenolic compounds, and more (Hou *et al.*, 2013). The main molecules in orange peel extracts include phenolic colorants and pectin (El-Rahman *et al.*, 2019). Orange peel is a significantly important source of dietary fiber, accounting for around 30% of the total weight of the fruit. Dietary fiber is a collection of carbohydrate polymers or oligomers that escape digestion in the small intestine (Fuller *et al.*, 2018). Different types of vitamins are also found in oranges such as Vitamin A, Vitamin B1, Vitamin B2, and Vitamin C. *Citrus* species are gaining more importance in controlling life-threatening diseases. Orange peel is the source of important compounds such as sugars and many acids that cause alteration in the soil acidity and influence photosynthesis and other processes of plant growth. Orange peel has a higher antioxidant activity than other parts of the fruit (Manthey and Grohmann, 2001). Orange peel regulates the soil PH and also contains important nutrients such as Phosphorus, Sodium, and Potassium which are needed by soil as a fertilizer (Jariwala and Syed, 2016). The orange peel contains a large amount of important compounds such as N, P, and K which increase soil fertility and productivity (Panwar, 2015). The extract of orange peel can be applied to the cotton plant in order to improve its functional properties such as antimicrobial activity (Wolela, 2020), UV protection (Hou *et al.*, 2013), and also mosquito repellent properties.

These days chemical and biological processes are the most frequent methods for making nanomaterials. Despite the effectiveness of chemical methods, the synthesis of nanomaterials is currently restricted due to their negative environmental consequences (Yonezawa, 2018). Various chemicals used in the control of plant diseases contain hazardous compounds. As these chemicals are released into the environment, they offer a huge threat to all of us, prompting more studies into eco-friendly natural agents (Anwar and Alghamdi, 2020; Ragab and Hassabo, 2021).

Biological nanomaterial synthesis methods, such as producing metal nanoparticles using species from living organisms, are known for being environmentally friendly with no negative impacts on the environment

(Bonnia *et al.*, 2016). Plant extracts are environmentally acceptable materials that have gotten a lot of attention for the development of nanoparticles among various biological species (Maqbool *et al.*, 2021). Because orange peel extract contains several active phytochemicals that can be utilized as a reducing and stabilizing agent, it can be employed as a reducing agent for the manufacture of nanoparticles such as silver and zinc oxide nanoparticles (Anwar and Alghamdi, 2020). Nano-biotechnology is a rapidly expanding study topic in the twenty-first century, with several applications in a variety of scientific domains.

Nano fertilizers are a novel concept that is less harmful, more cost-effective, and provides balanced nutrition for crops to achieve full yield productivity, contributing in achieving a sustainable agriculture (Nisar *et al.*, 2019). Encapsulated nanoparticles improve the effectiveness of applied fertilizers, restore plant health, and simultaneously minimize soil toxicity, agroecological degradation, and pollution (Dimkpa and Bindraban, 2016). As stated, there are several advantages to using Nano fertilizers, making them an important part of the agricultural system. Nano fertilizer has the ability to boost NUEs while also assisting in the management of nutrient content throughout the crop-growing process. Nano fertilizers control the amount and rate of encapsulated fertilizers/nutrients, allowing for greater plant absorption. It enhances the availability of nutrients as well as their actual supply duration. Furthermore, because nutrients are released at a slower pace, there is less loss due to run-off and leaching, which reduces fertilizer demand in the long term. Nanoparticles are smaller than the pore size of leaves and roots, allowing vital nutrients to penetrate deeper into the agricultural plant. Nano fertilizers are also linked to improved health and nutrition (Zulfiqar *et al.*, 2019). It was determined that by increasing the availability of nano/micronutrients, the plant can be protected from deficiency of nutrients, illnesses, and abiotic and biotic stress. This aids in the improvement of the quality of agricultural crops and attaining a higher quality of food for both human and animals use. Another advantage of nano fertilizer over conventional fertilizer is its small size and capacity to distribute insoluble nutrients (Shang *et al.*, 2019). The use of nano fertilizers in crops by foliar application is a very successful technique of delivering necessary nutrients directly to the plants, and it may be done even under difficult soil conditions. Foliar

application is a prominent agro-technique that has a number of benefits over soil application, in which nutrients are absorbed into the soil particles. The application's quick nutrient absorption allows for rapid rectification of a variety of nutrient-related deficits that might otherwise degrade the production and quality of targeted crops (Alshaal and El-Ramady, 2017). Nano fertilizer foliar application has a significant impact on the growth, production, quality, and other physiological processes of the plant. The use of a di and tri foliar application of N, P, and K nano fertilizers has a significant influence on the growth and yield of wheat crops when compared to control conditions (Al-Juthery and Saadoun, 2018).

The aim of our present research was to investigate the presence of nanoparticles in orange peels and utilized it to increase the germination% and enhance the growth of *Vigna radiata* plants by reducing the infection % of root rooting fungi.

MATERIAL AND METHODS

Collection of Oranges

Healthy and fresh oranges were collected from the local



Figure 1. Fresh and healthy orange's peel and dry powder.

Formation of Nano fertilizer

About 500g of fresh oranges were washed in tap water and peels were separated into small pieces. The peels were then ground to a soft slurry in a blender and proceeds it according to the method described by Hussain and Hussain (2015), and further characterization was done by using TEM, XRD analysis and phytochemical screening.

Transmission Electron Microscope (TEM)

The orange peel extracted fertilizer that contains the nanoparticles were identified and measured by means of a transmission electron microscope (TEM). To prepare the TEM sample, it was dispersed in distilled water using sonication for duration of 10 minutes. Next, a small drop

markets of District Haripur Khyber Pakhtunkhwan.

Preparation of Methanolic extract from Orange Peel Powder

The oranges were thoroughly washed with running tap water in order to remove dust and other impurities. The peels were separated from oranges and placed on newspaper to dry it in the shade for 20-25 days at room temperature. Dried peels were ground in an electric grinder 2-3 times until it was converted into powdered form to make the process of extraction more efficient as the finer the orange peel powder the more extraction of active constituents would be possible from the peels. The powder was packed in polyethylene bags and labelled with specific codes (Figure 1).

About 100 grams powder of orange peel was added in 300ml of methanol and transferred to the flask and kept for 4-5 days with random shaking to allow the particles to fully dissolve. The infusion was filtered through the Whatman filter paper. After 24 hours the residue was extracted with an equal volume of solvent. The filtrate was concentrated through the rotary evaporator and was dried at 50°C on the rotary evaporator and used in different experiments for phytochemical screening.

of the dispersed sample was carefully placed onto a 400-mesh copper grid that had been previously coated. The grid was then left to dry naturally in an open-air environment at room temperature. To determine the average diameter of the nanoparticles, observations were made in multiple selected areas of the enlarged microphotographs. The analysis focused on nanoparticles within the range of 100nm.

XRD Test

The preparation of nano fertilizer using orange peel extract were studied by XRD using a diffractometer (Germany) having CuK α ($\lambda= 1.5406\text{\AA}$) radiation source and working at 40KV and 40 mA in 2 θ range of 10-60°C temperature.

Phytochemical Screening

The methanolic extracts of orange peel were screened for the presence of different types of bioactive phytochemicals such as alkaloids, steroids, quinones, polyphenols, terpenes and saponins.

Phenols

The test for the presence of phenol in the orange peel extract was performed by taking aliquots extract in a 10ml test tube and volume was increased up to 3ml by adding 3ml distilled water. This step was followed by adding 0.5ml Folin Ciocalteu reagent and 2ml Na₂CO₃ in a test tube. The phenols undergo redox reaction with phosphomolybdic acid in Folin reagent so blue color was appeared. The solution was heated for 1 minute on spirit lamp, it was cooled, and its absorbance was measured at 650 nm against the reagent which was used as a blank. Then a calibration plot was generated by using unknown concentration of catechol at 650nm. Through this calibration plot the concentration of phenol in test sample was determined and expressed in term of mg catechol that was equivalent of phenol/g of sample (Makinde *et al.*, 2015).

Phlobatannins

For determination of presence of phlobatannins 0.5g of orange peel extract was heated with 10% HCL on spirit lamp in boiling water, and observed (Makinde *et al.*, 2015).

Steroid

In order to test the presence of steroids 1ml of concentrated H₂SO₄ was mixed with 1ml of orange peel extract .The solution was allowed to settle in the test tube for 5 minutes (Makinde *et al.*, 2015).

Triterpenes

For testing the presence of triterpenes about 20mg of orange peel extract was mixed with 10ml chloroform. The solution was heated on a water bath and then filtered using filter paper. After that 5ml concentrated H₂SO₄ was added and mixed in the chloroform filtrate. The mixture was observed (Makinde *et al.*, 2015).

Xanthoproteins

About 1ml of extract was taken in a test tube and a few drops of nitric acid were added. After that ammonia solution was added drop wise (Makinde *et al.*, 2015).

Utilization of Formulated Nano Fertilizer from Orange Peels in Screen House Experiment

A screen house pot experiment was carried out to check the effect of nano fertilizer prepared from the orange peel powder with a complete randomized block

designed. Considering the proper effect of light ensures that soil must have a heterogeneous population of pathogens in it. Sandy loam Soil having pH of 7.5 obtained from the experimental plot of Department of Botany, Women University Swabi and transferred in 15cm diam plastic pots, each pot consists of 780gm soil and replicated by 3 times. The soil was naturally infested by soil pathogens of 4-7 sclerotia of *M. phaseolina*/gm of soil, 2000cfu/gm of combined population of different species of Fusarium, and 3.5% colonization of *R.solani*. Fungicide (Metalaxyl + Mancozeb) was used as positive control and comparative analysis with treatments, while 1%w/w, 3%w/w and 5%w/w orange peel powder were mixed with soil as treatments effect. While without treated pots were considered as negative control that were also used for comparison. All the pots were kept at 50%water holding capacity in green house for a period of one week. Ten seeds of *Vigna radiata* were sown in each pot and after 7 to 8 days of germination, only five were kept in each pot and extra were removed. Observations were recorded after 45 days of seedling of plants. After that period plants were uprooted and washed with running tap water. Fresh growth of plants and incidence of fungi were recorded.

Isolation of Fungi from Roots of *Vigna radiata*

After uprooting the roots were washed under running tap water and surface sterilized with 1% Ca(OCl). The sterilized roots were cut into 1cm long pieces and were placed on PDA in a petri dish containing penicilin (100000 units/liter) and streptomycin (0.2gm/litre) and incubated at 28 °C for 5 days to check the infection and colonization percentage of soil borne fungi. Infection percentage and frequency of colonization were calculated as follows:

$$\text{Infection \%} = \frac{\text{No. of plants infected by a pathogen}}{\text{Total no. of plants}} \times 100$$

$$\text{Colonization \%} = \frac{\text{No. of root pieces colonized by a pathogen}}{\text{Total no. of root pieces of all plants}} \times 100$$

Data Analysis

The data were analyzed and subjected to analysis of variance (Anova) which was followed by the least significant difference (LSD). All analysis was performed using IBM SPSS STATISTICS program (Sokal and Rohlf, 1995), Particles size of nano fertilizer was calculated by "Image.J free software" Program by Joonas "Regalis" Rikkonen, version 1.8.0.

RESULTS

In vitro Phytochemical Screening

The result of in vitro phytochemical screening of orange peel powder showed the existence of phenols, steroids and triterpenes while phlobatannins was not observed in the aqueous extract. The aqueous extract was yellow, pungent and soft. The appearance of blue colour indicated the presence of phenol. No phlobatannins were observed in the extract of orange peels because no red

color was appeared as an indication of these phytochemicals. While appearance of brown color indicated the presence of steroids. When a test for triterpenes was performed the presence of reddish precipitates indicated the presence of triterpenes in the extract. While during the test of Xantho proteins formation of brownish and reddish precipitates were observed which indicated the presence of Xanthoproteins (Makinde *et al.*, 2015) (Figure 2).

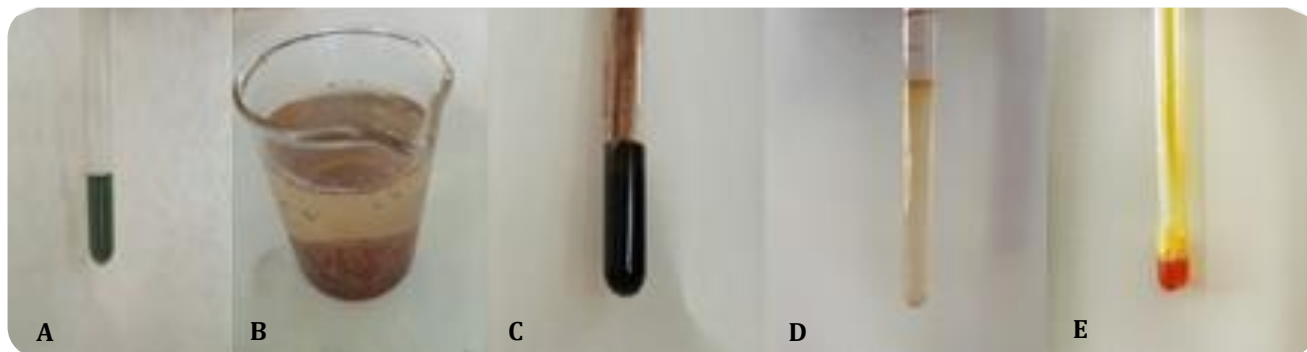


Figure 2. A= Indicated Presence of Phenol, B= Absence of Phlobatannins, C= Presence of Steroids, D= Presence of Triterpenes, E= Presence of Xanthoprotien.

Screen House Experiment

The experiment was carried out to check the efficacy of orange peel powder on the root and shoot growth of mung bean plant. The observation was recorded after 45 days. Present experiments showed that the organic amendments of orange peel powder act as a nano fertilizer to enhance growth parameters of *Vigna radiata*. (Figure 3, Table 1). The result reveal that application of nano fertilizer at 1, 5 and 10% showed significant result compared to control in root length that were recorded 8.55cm, 9.66cm and 6.88cm

respectively, while 18.44, 16.11 and 19.10cm shoot length were recorded at 1, 5 and 10% that was significant result compared to +ve and -ve controls. (Table 1, Figure 4).

Effect of Nano fertilizer to Reduce the Infection Percentage of Root Pathogenic Fungi

Infection % of *Fusarium solani* in *Vigna radiata* completely suppressed in the 10 percent treatment of orange peel powder at ($p < 0.05$) compared to +ve and -ve control. Whereas the infection% in (1% and 5%) treatment was significant.

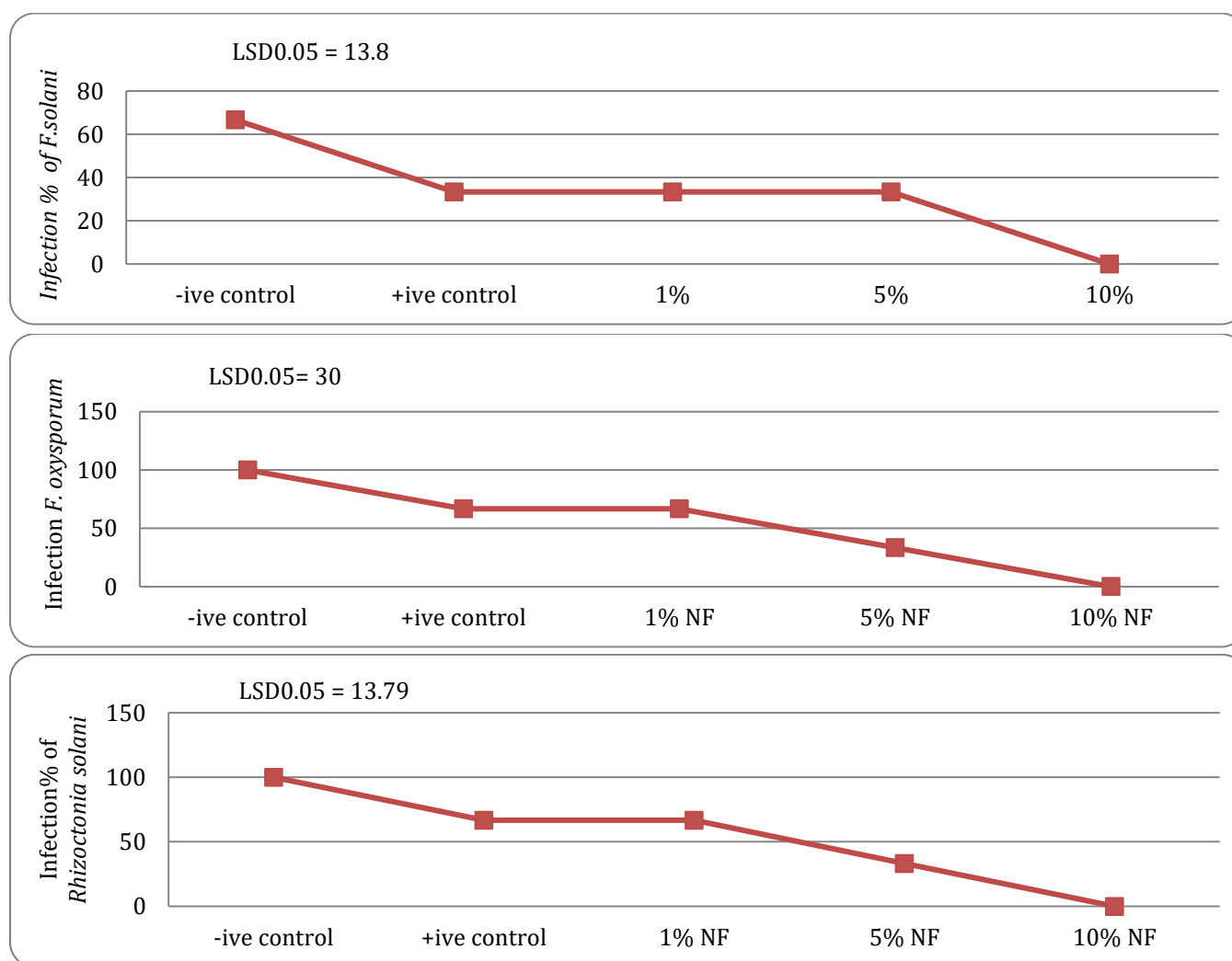


Figure 3. Comparison of growth of *Vigna radiata* after application of orange peel powder as a treatment with the different concentration.

Table 1. Effect of orange peel on various parameters like root length, shoot length root weight shoot weight and leaf area of *Vigna radiata*.

Treatment	Root length (cm)	Shoot length (cm)	Root weight (mg)	Shoot weight (mg)	Leaf area Cm ²
Metalaxyl+Mancozeb (Control +Ve)	6.88	16.11	0.04	0.58	5.95
Control-Ve	4.99	12.33	0.03	0.43	4.22
OPP (1% w/w)	8.55	18.44	0.07	0.50	8.15
OPP (5% w/w)	6.88	16.11	0.08	0.74	11.17
OPP (10% w/w)	9.66	19.10	0.17	0.82	19.59
LSD _{0.05}	0.471	0.29616	0.00682	0.04006	0.60790

OPP Orange Peel Powder

Figure 4. Infection % of different Pathogens isolated from the root of *Vigna radiata*.

Infection% of *Rhizoctonia solani* in orange peel powder treatment at various concentration was different in contrast to -ive control, whereas infection %age at 10% treated plants was totally suppressed as compared to +ive and -ive control ,while in (1% and 5%) treated

plants the infection % age significantly shown best results as compared to -ive control at ($p < 0.05$). Infection percentage of *Fusarium oxysporum* in 10 % treatment was completely suppressed in treatment of orange peel powder at ($p < 0.05$) compared to +Ve and -

Ve control. While the infection% in 5% treated plants was significant at ($p < 0.05$) compared to +ive and -ive control (Figure 4).

Characterization of Orange Peel Extract by TEM Analysis

The TEM analysis of orange peel extract showed at a resolution of 100nm in which nanosphericles particle structures ranged in size from 18 to 61nm (Figure 5, Table 2).

Result of XRD

The X-ray diffraction (XRD) pattern of nanoparticles synthesized using orange peel extract is illustrated in

Figure 9. Peaks were identified at 2θ values of 19.830, 20.550, 21.240, 24.440, 27.330, and 29.770, demonstrating a close match with urea peaks. The determination of particle size relied on the full width at half maximum (FWHM) of these peaks, as analyzed through the Scherrer formula;

$$T = \frac{K\lambda}{\beta \cos \theta}$$

Where, t crystallite size and λ = wavelength of diffraction radiation. The $k = 0.94$ [57] and β = line width at half-maximum height after deduction of particle size value of urea coated nanoparticles was 2nm (Figure 6).

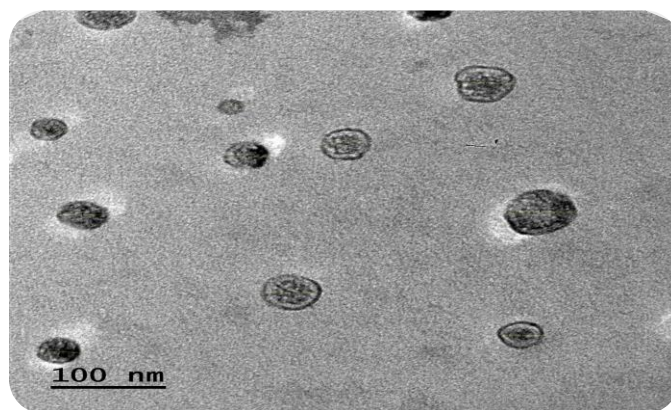


Figure 5. TEM analysis of orange peel extract at 100nm.

Table 2. Measurement of Nanoparticles of orange peel at 100nm.

Label	Area	Mean	Std Dev	Min	Max	Angle	Circ.	Length
Particle 1	7.355	46.873	51.648	0	216	-2.203	0.081	33.79
Particle 2	10.447	98.57	36.99	18	199	-3.094	0.057	48.12
Particle 3	13.258	98.198	41.769	0	215	-1.219	0.045	61.05
Particle 4	8.479	106.762	33.541	26	199	1.909	0.07	38.981
Particle 5	10.728	78.258	32.585	9	163	0	0.055	49.349
Particle 6	7.355	84.65	36.428	0	161	0	0.081	33.765
Particle 7	9.042	77.99	40.969	0	178	-1.79	0.066	41.577
Particle 8	3.982	118.6	34.712	48	200	-4.086	0.151	18.227
Particle 9	6.793	86.738	38.473	0	174	0	0.088	31.168
Particle 10	8.479	84.105	31.862	21	187	-1.909	0.07	38.981
Mean	8.592	88.074	37.898	12.2	189.2	-1.239	0.076	39.501
SD	2.529	19.49	5.875	16.116	19.82	1.756	0.029	11.681
Min	3.982	46.873	31.862	0	161	-4.086	0.045	18.227
Max	13.258	118.6	51.648	48	216	1.909	0.151	61.05

DISCUSSION

Using chemicals to control the growth of seed-borne and root-rotting fungi is an effective method but very costly. However, the use of organic matter is one of the best

approaches to suppress plant growth of organic matters is one of the best approaches to suppress the growth of the plant. In the present study, we used the extract of orange peel powder as a nano fertilizer to promote the

growth of *Vigna radiata* and to reduce the root rotting pathogen.

Nano fertilizers are made up of nanosized components that act as fertilizer carriers and vectors for controlled release which is why they are also known as smart fertilizers (Calabi-Floody *et al.*, 2018). Nano fertilizers are frequently employed in agricultural systems to

improve nutrient use efficiency (NUE) because of the unique features of nanoparticles. Coating fertilizer particles with nanomembranes allows nutrients to be released continuously and slowly over a long period of time, resulting in little mineral loss during crop fertilization and thereby providing balanced nutrition to crops (Shebl *et al.*, 2019).

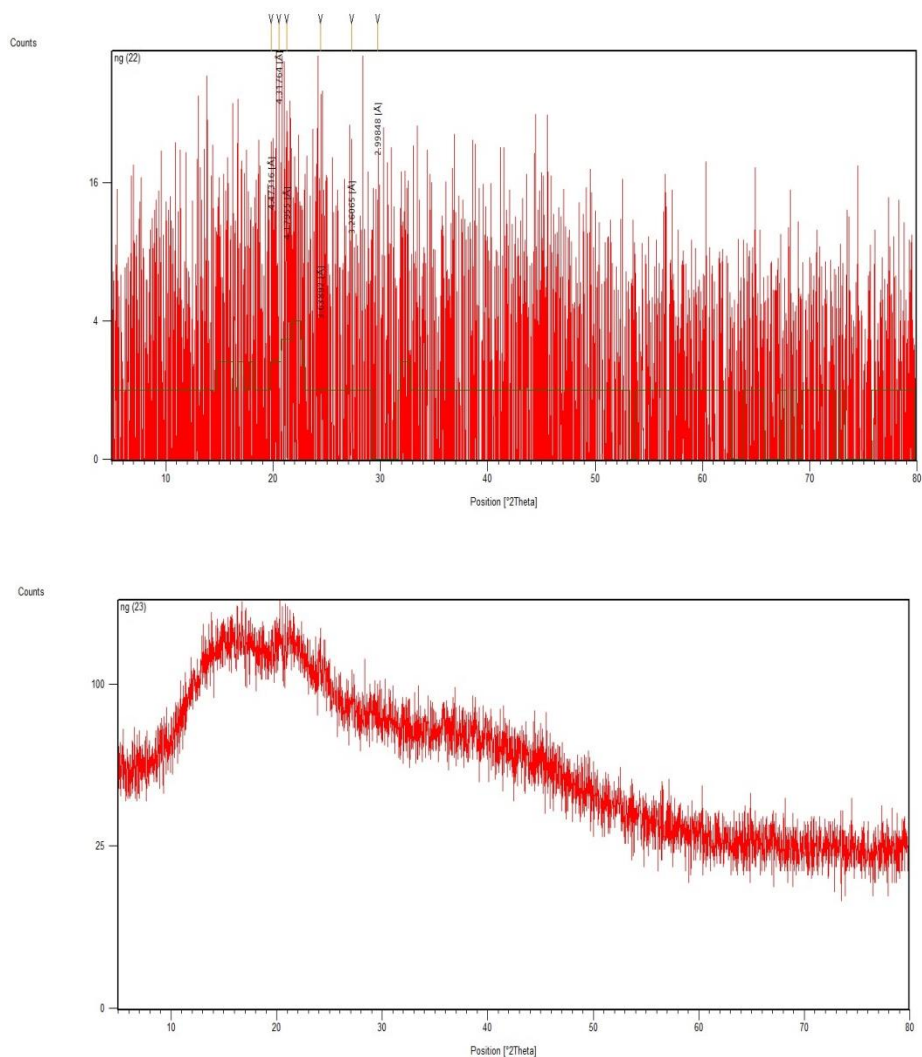


Figure 7. XRD pattern of the urea nanoparticles.

When orange peel extract coated with urea nanoparticles was applied as a fertilizer in *Vigna radiata*, it was observed that the germination percentage increased significantly as compared to the control sample. The results are due to the increased release of amino acids and potassium which act as growth promoters as mentioned by Emaga *et al.* (2007). These authors investigated the effect of tryptophan on the

growth of plants such as wheat and periwinkle. These regulated the growth of plants by improving the physiological activity and water availability of plants under water stress conditions that resulted in the better uptake of nutrients. Similarly, in the case of banana peel extract, the tryptophan treatments resulted in increasing endogenous indole-3-yl-acetic acid (IAA) and phenol and total free amino acids (Bakry *et al.*, 2016).

Present experiments showed that the organic amendments of orange peel powder coated with urea nanoparticles act as a nano-fertilizer to enhance growth parameters of *Vigna radiata*. The aqueous extract of orange peel powder contains various phytochemicals they are effective for growth and development of plants. The experiment was carried out under field conditions to check the growth of *Vigna radiata*. The plant with organic amendment was healthier as compared to control because orange peel powder acted as a nano-fertilizer. It has also been found that plants grown under high concentration of orange peel amendment shown lesser infection and colonization percentage of root rotting fungi as compared to positive and negative control. Taha *et al.* (2020) applied banana peel extract and concluded that it has antimicrobial and antioxidant activity. Similarly, it was found that the use of urea and citric acid are involved in liberating minerals, amino acids, tryptophan, and total protein. The presence of these liberated materials has great effects on the plant germination (Aboul-Enein *et al.*, 2016).

Based on our present work it was concluded that potassium hydroxide was used as a dissolving agent for cellulosic components and urea coated nanoparticles that resulted in the liberation of fertilizing agent in the sample solution followed by the addition of carrier and chelating agents that resulted in the synthesis of bio stimulant nano fertilizer. Different types of phytochemicals were observed in orange peel extract that showed significant activity when applied to enhance the growth of *Vigna radiata* and to suppress the root rotting pathogens. XRD test also confirmed the presence of urea coated nanoparticles compared the peaks observed.

CONCLUSION

In this study, orange peel extract was prepared from dried orange peel powder and analyzed through phytochemical screening, showing the existence of different phytoactive compounds. Such as phenols, steroids, triterpenes, and Xanthoproteins. Then the characterization of nano fertilizer was performed through a transmission electron microscope (TEM) which revealed the presence of various nanoparticles that ranged in size from 18.227 to 61.05nm at a resolution of 100 nm. The XRD pattern of nanoparticles using orange peel extract were shown in fig 9. The peaks were observed at 2θ value of 19.83° ,

20.55° , 21.24° , 24.44° , 27.33° and 29.77° which was well matched with the peaks of urea. Similarly, the nano fertilizer was applied to check the growth of *Vigna radiata* under a screen house experiment that not only enhanced the growth parameters of plants but also suppressed the population of root-rotting fungi, thus acting as a cheap and effective method to improve the growth and development of the plant in the field of agriculture.

STATEMENTS AND DECLARATIONS

Authors affirm that there are no financial or non-financial conflicts of interest.

AUTHORS CONTRIBUTIONS

Saba Iqbal: Conducted Research

Gulnaz Parveen: Conceived idea of research, Supervised and wrote the paper

Ambreen Ayub: Helped to do the Experiment of XRD

Salma Gul: Helped in Phytochemical analysis

Tahira Batool: Analyzed data

Nain Tara: Helped to Identify the Pathogens

Amtul Sami: Wrote Introduction

Atiya Hussain Khowaja: Over all technical support.

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