GMR

The Original

# ZINC OXIDE BIO FABRICATED NANOPARTICLE: METHODOLOGY, CHARACTERIZATION AND IT'S *IN VITRO* EFFECT ON FALSE SMUT OF RICE [*USTILAGINOIDEA VIRENS* (COOKE)TAKAHASHI]

**Dr. P.P. Khandagale[1] *, Dr. P.R. Patel[2], Prof. B. M. Mhaske[3], Dr. J. J. Padsala[4], Dr. S. B. Gawade[5]**

[1]Assistant Professor, Wheat Pathology, Agricultural Research Station (MPKV), Niphad, Dist. Nashik. Maharashtra
[2]Head, Department of Plant Protection, ASPEE College of Horticulture, NAU, Navsari.Gujarat
[3]I/C. Wheat Specialist, Agricultural Research Station (MPKV), Niphad, Dist. Nashik .Maharashtra
[4]Assistant Research Scientist, Potato Research Station, S.D. Agricultural University,Dessa ,Gujarat.
[5]I/C.AICRP on Medicinal Aromatic Plants and Betelvine, M.P.K.V., Rahuri, Dist.Ahilynagar, Maharashtra
*Corresponding Author: Dr. Khandagale P.P, E mail: p2khandagale@gmail.com

## ABSTRACT

Rice (*Oryza sativa* L.) is a major staple food grain crop provides nutrition to more than half of the world. The new era of science with utilization of different nanoparticle for human benefits was well known. Though nanoparticle were synthesized by physical, chemical and physiochemical method plant mediated - fabricated metal nanoparticle was one of the promising alternatives having safe, eco -friendly, simple, cost effective and nontoxic product at final. Research focuses on the green bio fabricated methods of Zinc oxides nanoparticle formation by using garlic bulb (*Allium sativum*) extract and zinc sulphate. The UV-Visible spectroscopy (UV-Vis) at 358 nm, Fourier Transform–Infrared Spectroscopy(FT-IR) range at 400-4000 $cm^{-1}$, with metal oxide nanoparticle formation takes place at less than 500 $cm^{-1}$ and Scanning Electron Microscopy (SEM) reading ranges from 48 to 89 nm with spherical agglomerate shapes confirmed the nanoparticle formulations. Under *in vitro* green ZnO NPs tested for their ability to inhibits the rice false smut [*Ustilaginoidea virens* (Cooke)Takahashi] fungal growth at its five different concentration with three negative fungicidal control (Trifloxystrobin 25% + Tebuconazole 50% WG) and one standards control. Result shows that green synthesized ZnO NPs was control the rice false smut at sustainable level with 100 ppm concentration with 64% inhibition than respective fungicidal concentration pointing to their use in plant disease management with environment friendly, safe, cost effective having novel approach.

**KEYWORDS**-Green nanotechnology, false smut of rice, ZnO NPs

## INTRODUCTION

Rice plays a pivotal role in food and nutritional security for more than half of the world's population (Bao, 2020).The global production of rice has been estimated at 503 million MT and area under rice cultivation is estimated at 165 million hectares (Anonymous, 2022 ;Statistica, 2023).Where India contributes 21 per cent of global rice production. Population explosion and food security pressurized for the cultivation of semi dwarf, high nitrogen responsive varieties along with imbalanced use of inputs like fertilizers and pesticides, dense planting and crop canopy accelerated the disease and insect problem during the past high yielding varietal era (Karmakar *et al*. 2016).Though rice is a host, more than 70 diseases causes' significant damage to rice crop in India. The false smut of rice smut caused by [*Ustilaginoidea virens* (Cooke)Takahashi] (teleomorph *Villosiclava virens*, Tanaka,2008) ( Lu *et al*. 2009; Manu *et al*. 2017), is became minor to major due to global warming and changing cultivation practices .Rice false smut or pseudo smut was first described by Cooke in 1878 from Tirunelveli, Tamil Nadu (Laha *et al*. 2016) in India having an incidence up to 50% with percentage of infected tillers was 10–20 per cent and 5–85per cent in Punjab and Tamil Nadu respectively (Ladhalakshmi *et al*. 2012;Wang *et al*. 2019). The disease is characterized by the replacement of rice grains into yellow false smut balls which later on converted to dark green smut balls scientifically called psudomorph. The yield loss due the false smut infection on rice varies with state wise agro climatic zone and cultivar which were cultivated (Masurkar *et al*.2023) like highly aromatic and high yielding variety Pusa Basamti-6, JRH-5(Utter Pradesh) with 57.11 and 53.08 percent yield loss respectively. The disease also affect rice seed yield and grain quality by their chaffiness (24.6–87.5%)with simultaneous reduction in filled grains (3.4–71.8%) and decrease in 1000 grain weight by 1.2–10.8 per cent (Srivastava *et al*.2014). Seedlings vigor was negatively affected when emerged from infected panicle-grain (Bag *et al*.2016).Though the pathogen was managed by different chemical and cultural methods but they leads to soil and water pollution with toxicity to bio sphere . The garlic (*Allium sativum*) the well-known culinary vegetable in kitchen of every Indian having antimicrobial properties used in traditional medicine (Goncagul and Ayaz, 2010 and Strika *et al*.2017).Zinc is the popular anti- fungal substance used to control many fungal disease from ancient time. Zinc oxide is an in organic compound had increased surface area and activity of its nano scale size, they enhances the efficiency of aqueous and organic solvent allowing them for incorporation into material process. Plant-based or bio fabricated green synthesis NPs now regarded as a gold standard technique(Khan *et al*.2022) owing to its ease of use, biocompatible, inert, ecological friendly analytical chemistry and methodologies (Malik *et al*.2014). The green nanotechnology is one of the emerging sustainable aspects applied for disease management in agriculture for residue free and hygienic harvest. The ZnO NPs from garlic extract have wider adaptability in nanomaterial synthesis and application for every beneficial aspect of human being (Naseer, 2020). Here we prepared the zinc oxide nanoparticle by using garlic and find its effect for management of rice false smut disease *in vitro* after their characterization.

## MATERIAL AND METHODS

### 1. Methods for preparation of green Zinc oxide nanoparticle

#### a. Methods for preparation of garlic bulb extract-

Local good quality garlic was purchased, separated, cleaned and take each single clove without soil debris, wash it with distilled water thoroughly without peeling its outer skin. According the protocol given by Soloman *et al.* (2023), of garlic extraction for preparation of ZnO NPs bulbs was take completely with its moisture contents, crushed thoroughly using clean mortar and pestle. When the crushed bulb showing watery contents in the mortal take 60 g of the crushed mixture - powder of sample in separate clean beaker container and mixed it with 200 ml deionized water. Than the sample in beaker placed in heating chamber boiled at $60^0$C for 30 min. When the yellowish golden black filter color was appeared stop the boiling. Finally, the extracts were filtered with the What man No. 1 filter paper and take it freshly for further synthesis of ZnO NPs. No any preservation required to filtrate so avoid its volatilization and loss of chemical constituents.

#### b. Methods for preparation of garlic Zinc oxide nanoparticle (ZnO NPs)

The Zinc sulfate heptahydrate ($ZnSO_4, 7H_2O$) as zinc precursor and sodium hydroxide as alkalizing / precipitant medium used for the green preparation of Zinc nano particles. Freshly prepared garlic extract as above were used acting as capping agent. The deionized water was used as solvent for the preparation of all procedure arrangements. Garlic extract is used to reduce Zn ions toxicity with same time allicin and other sulpher derived phytochemical present in them stabilizes nanoparticles. The garlic bulb extract was acts impregnate ZnO NPs or as a solvent for synthesizing nano bio hybrids (Kumar and Venkateswarlu (2013); Aldalbahi *et al*. (2020) and Soloman *et al*.(2023).

#### i. Nucleation of solid Zn O NPS

The 40 ml of pre prepared garlic bulb extract in new beaker and pour slowly to the beaker containing Zinc sulphate at 50 ml concentration with constant stirring (0.1 M)(Alami *et al*.2015).

#### ii. Precipitation and collision of Zn O NPS

The 1 M NaOH (100 ml) the precipitation agent were added drop by drop to that mixture with stirring for less than hour with controlling the pH at 9.(Herrera -Rivera *et al*. 2017,Solomon *et al*,2023 and Hasan *et al*,2018). Here under the high concentration of hydroxyl ion in a solution intermediate product like as Zinc hydroxide $(Zn\ OH)_2$ and salt containing tetra hydroxozincate ion are formed $(Zn\ OH_4)^{2-}$ ).

#### iii. Growth and ripening of Zn O NPS

Then put solution beaker on hot plate magnetic stirrer for 2 h at the temperature $50^0$C until the white brown precipitate was observed. The mother liquor on top was evacuated. The white precipitate was washed several times with deionized water which was now assumed to be $Zn\ (OH)_2$ free of impurity. Than precipitate was centrifuged three times for alternate 10 min interval at 5,000 rpm. Lastly the white brown powder obtained were oven dried at 50 $^0$C for 24 h.

#### d. Calcination and coarsening of Zn O NPs

Calcination involved extreme heat treatment to the material at controlled temperature where particle fuse and enlarge its primary crystalline size called particle coarsening (Ruys,2019).Which often leads to growth of larger crystal from smaller size and reduced it in smaller size while larger particle to grow. The calcination at higher temperature with 2 h leads to formation of smaller size ZnO NPs. The dried sample of ZnO nanoparticles was obtained by calcination temperature of $450^0$C for two hours in muffle furnace. The blackish-white brown powder was collected and preserved in air tight container and use it for further characterization. The XRD characterization according to the Scherer equation indicated that the crystalline size increased with increasing calcination temperature when ZnO NPs prepared from irrespective of different methods of synthesis of Zinc nanoparticle (Parra and Haque,2014).

### 2. Method for characterization of green ZnO NPs

The characterization of our green synthesized ZnO NPs by using different analytical methods like UV-Vis spectrophotometer (UV-1800 series), FT-IR spectra photometer, Scanning Electron Microscopy (SEM) and X- ray Diffraction (XRD), for the confirmation of qualitative and quantitative parameter resembles with nanoparticle production .

#### UV-Vis spectrophotometer

From the graph in UV-Vis , having absorbance verses wavelength obtained by running the 1 ppm garlic ZnO NPS dissolving with distilled water in spectrophotometer caveat and the control without sample was run on the same instrument.The best peak without any undulating topography shows purity of our prepared garlic ZnO NP s.

**Scanning electron microscopy (SEM)**
The surface morphological characterization and grain particle size of synthesized ZnO nanoparticles was determined by Scanning electron microscopy (SEM). The surface morphology and size of the garlic synthesized ZnO NPs was imaged. The 10 ul of ZnO NPs were paced in lacey carbon supported nickel grids and examined using a high resolution SEM having size at an average lower than 100 nm (Jari *et al*.2022).

**Fourier Transform Infrared (FT-IR) spectroscopy**
By measuring the vibrational frequency of the bonding within the functional group the spectroscopic techniques enables us to identify the functional groups that are present on the surface of ZnO NPs. Used instrument Shimadzu- 8400S with ATR facility with scan range 400-4000 $cm^{-1}$. In our research the garlic synthesized ZnO NPs particle obtained having FT-IR ranges from $400^{-1}$ to 4000 $cm^{-1}$ (Jari *et al*. 2022).

## 3. METHODS FOR ISOLATION, PURIFICATION AND *IN VITRO* ANALYSIS OF EFFECT OF GREEN ZNO NPS ON FALSE SMUT OF RICE

The typical rice false smut diseased sample samples were collected from the Hill Millet Research Station (HMRS),Waghai, Dangs, Navsari Agricultural University, Navsari. The pathogen was isolated from the infected rice panicle, by adopting the standard tissue isolation laboratory techniques with slight modifications wherever necessary.Small pieces of symptomatic smut ball sample were cut from the diseased black smutted boll .Are surface sterilized with 0.1 per cent sodium hypochlorite solution for 60 seconds. These bits were thoroughly washed in sterile distilled water to remove the traces of sodium hypochlorite 2 to 3 times. The surface sterilized diseased pieces were transferred to pre autoclaved and sterile Potato Sucrose Agar (PSA) plates and incubated at room temperature (27 ± $2^0$C) for 10-15 days in dark condition (Young *et al*.2017) by rapping the petri plate by newspaper placed them in dark box in incubator BOD . The plates were observed periodically for fungal growth and sporulation. After twelve to fifteen days of incubation, the fungal growth was transferred aseptically to fresh PSA slants and incubated at 27 ± 2ºC for further use. Further purification was carried out by the single spore isolation technique and maintained the pure culture. Primary and filed crop identification of isolated fungi shows false smut disease appear at late flowering to grain formation stage continue near to harvesting stage. They were abnormal rounded, greenish, blackish ball like structure was observed attached to the panicle of plant. The growth is intercellular bio trophic, non- haustoria, all the stamen, stigma, lodicules and coleoptile of grain and epidermal cells of seedling roots showing the growth of mycelium (Young *et al*.2017; Tang *et al*.2013).No infection found on leaves stems, pedicel of rice plant.
*In vitro* evaluation of antifungal property of green synthesised ZnO NPs was carried by employing poisoned food technique at five different concentration(10,25,50,75 and 100 ppm)with one control and negative control of fungicides Trifloxystrobin 25% + Tebuconazole 50% WG at 100,200 and 400 ppm concentration. The work was placed with CRD (completely randomized design) design.
All the nanoparticles were firstly dissolved in distilled water in beaker with magnetic stirrer at 1500 rpm for 5 min. All dissolved nanoparticles were mixed with PSA using poison food techniques (Schmitz, 1930) under *in vitro* condition with requisite concentration from the stock solution prepared at 1 mg in 1 lit of distilled water by using the formula of $N_1V_1=N_2V_2$. All the procedures were followed as used in evaluation of fungicides on test pathogen. Three replicates were maintained in BOD incubator at 27±$2^0$ C for 8 to 14 days observation was taken to avoid saprophytic contamination.

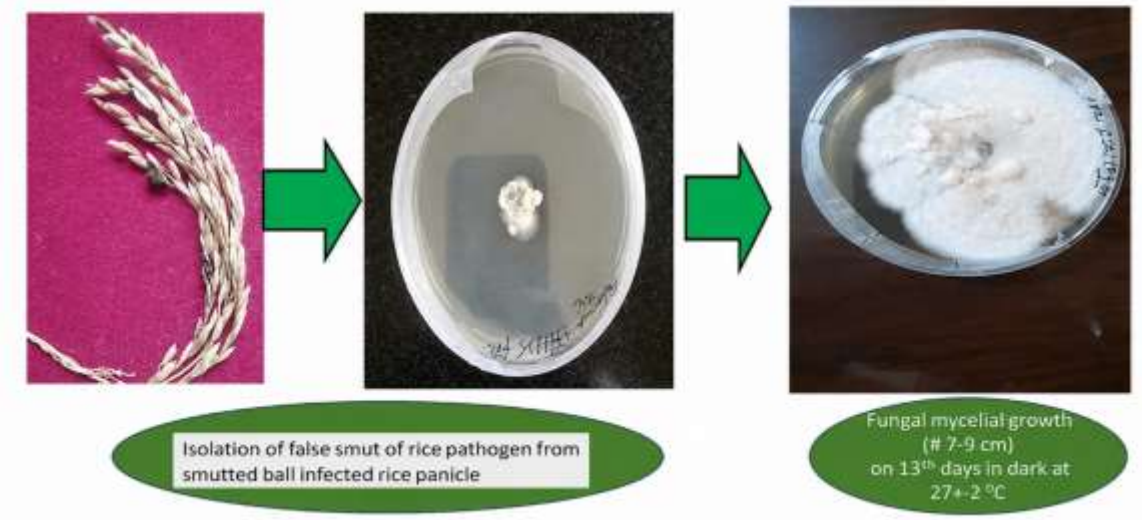



**Photo :1. Rice false smut ball infected panicle, isolation and growth of pathogen**

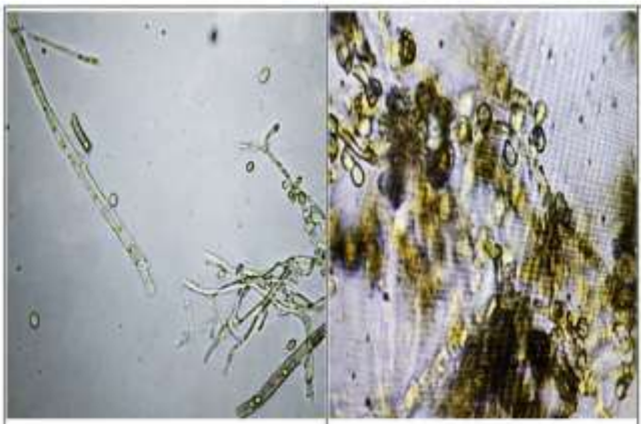


Photo :2.Microscopic observation of false smut of rice pathogen mycelia and conidiospore

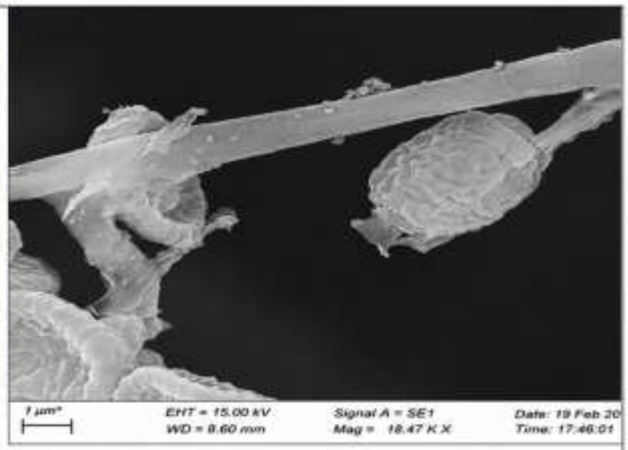



Photo :3. Scanning Electron Microscopic image of single conidiospore

The observations on mycelial growth (cm) and per cent growth inhibition of pathogen was recorded after 8 days of incubation. Though the growth of the pathogen cloddy, puffy whitish brown to greenish violet in the petri plate (photo 1,2 and 3) all having same spore shape, size and structure colour at microscopic identification. The per cent growth inhibition (PGI) of pathogen in each treatment will be calculated according to formula given by (Vincent, 1974).

$$PGI = \frac{DC - DT}{DC} \times 100$$

Where,
PGI = Per cent growth inhibition
DC = Average diameter of mycelial colony from control set (cm.)
DT = Average diameter of mycelial colony from treated set (cm.)

The data collected from laboratory experiments were subjected to rigorous statistical analysis using the analysis of variance (ANOVA) technique, as outlined by Panse and Sukhatme (1985).

**RESULT AND DISCUSSION**

Prepared the green zinc oxide nanoparticle by using the garlic bulb extract and zinc sulphate. The fig.1 shows the graph without undulation at 358 nm size of green ZnO NPs.

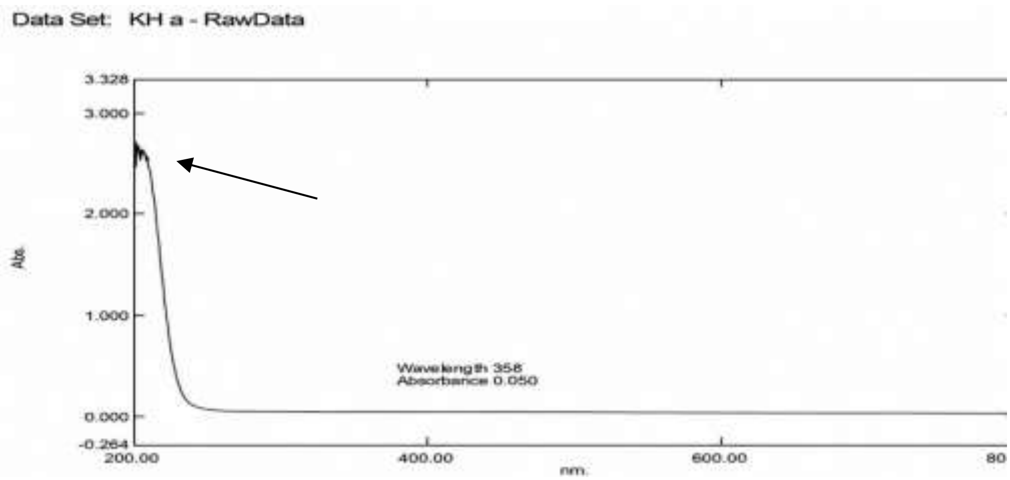



**Figure 1.UV-Vis spectrophotometer graph**

The green ZnO with different zinc acids precursor also gave same tune like us (Ali and Ali, 2023) with 300 nm size, Soloman *et al*. (2023) in garlic bulb and Zinc acetate with ZnO NPs at 369.5 nm UV-Vis peak, Benshiga *et al*.(2024) in their ZnO NPs by mixture of garlic extract and zinc nitrate gives203-409 nm UV-Vis size range. Modi and fulekar (2020) in their nanoparticle from mixture of garlic skin extract and zinc chloride UV-Vis range at 200-800 nm and best peak at 375 in their ZnO NPs and Islam *et al*. (2024) in their finding of nanoparticle preparation with onion peel waste extract with Zinc acetate precursor had UV- Vis range at 280-450 nm and best peak meet at 322 nm.

**Fourier Transform Infrared (FT-IR) spectroscopy**

Measure the vibrational frequency of the bonding within the functional group by the spectroscopic techniques FT-IR. Which help to identify the functional groups that are present on the surface of ZnO NPs. The ZnO NPs particles having FT-IR ranges from 400 $^{-1}$ to 4000 cm $^{-1}$.

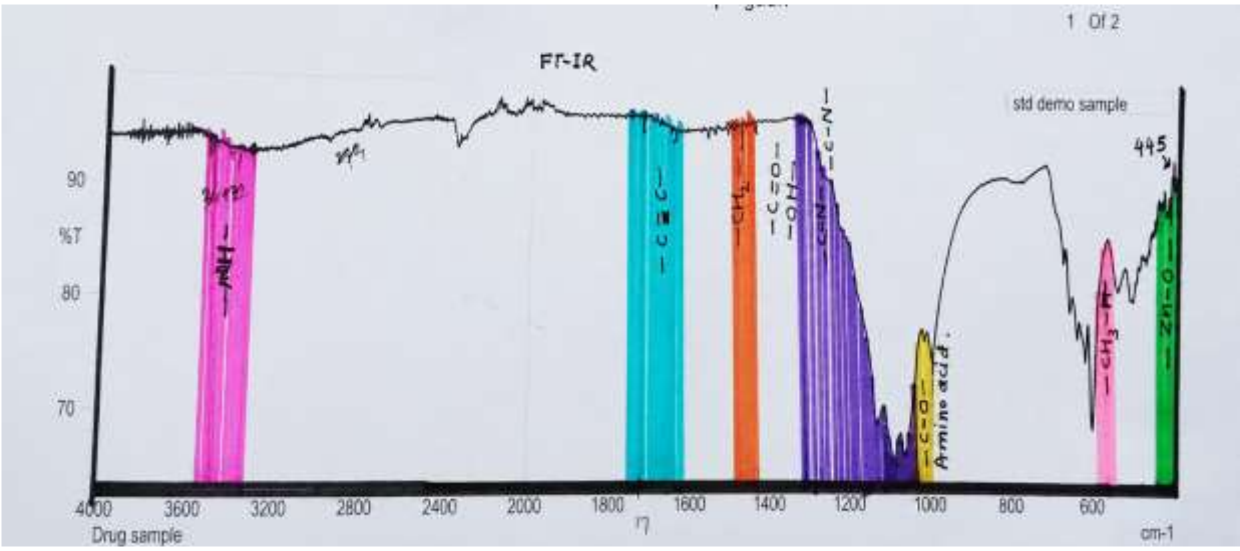



**Figure 2. FT-IR graph of synthesized green Zn ONPs**

The first broad peak at 443 cm $^{-1}$ less, than 500 cm$^{-1}$ indicating the formation of metal oxide (Zn ONPs ) formation(fig.2)associated with Jari *et al*.(2022) finding. The peak at 530 cm $^{-1}$ to 570 cm $^{-1}$ had metal oxide group may with alkyl halides groups. The band stretching at 1020 cm $^{-1}$ -1050 cm $^{-1}$ resemble with amino acid -C-O- banding with carboxylic acid deformation. The band stretching at 1370 cm $^{-1}$ -1400 cm $^{-1}$ pertain with -C=O- and -OH- banding. The band stretching at 1000 cm $^{-1}$ -1350 cm $^{-1}$ with presence of -C-N- group , band range 1440 cm $^{-1}$ - 1470 cm $^{-1}$ resemble with -$CH_2$- banding vibration and band at 1650 cm $^{-1}$ to 1760 cm $^{-1}$ resemble with -C=C- alkenes aromatic ring present. The band stretching at 3300 cm $^{-1}$ - 3500 cm $^{-1}$ with presence of -NH- band.

The same tune was found in different reference as Ali and Ali(2023),Benshiga *et al.*(2024) in their ZnO NPs by mixture of garlic extract and zinc nitrate found FT-IR ranges from 400 $^{-1}$ to 4000 cm $^{-1}$.Modi and fulekar (2020) found in green ZnONPs, FT-IR analysis ranges from 400 $^{-1}$ to 4000 cm $^{-1}$ and ZnO bonding peak at 441 cm $^{-1}$ confirm the metal nanoparticle formation where as Soloman *et al*.(2023) in their green ZnO NPs had FT-IR same ranges from 400 $^{-1}$ to 4000 cm $^{-1}$and ZnO bonding peak at near 1000 cm $^{--1}$ .Islam *et al*. (2024) in their finding of nanoparticle preparation with onion peel waste extract with Zinc acetate precursor in different volume of deionized water had FT-IR ranges from 400 $^{-1}$ to 4000 cm $^{-1}$ in their ZnO NPs with ZnO bonding peak at 822.60 cm $^{-1}$ and 655.13 cm $^{-1}$ confirm the metal nanoparticle formation in two different condition like 50 gm onion peel waste in 100 ml deionized water and 50 gm onion peel waste in 200 ml deionized water respectively. All procedure and characterization in our study was resembled and related with all above previous references.

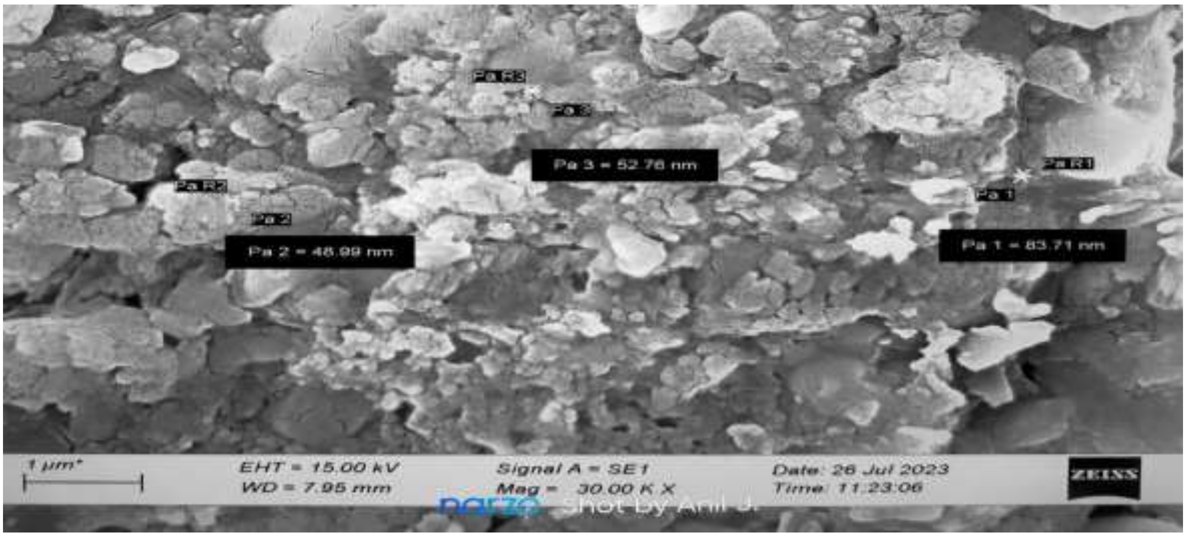



**Figure 3. Scanning electron microscopy analysis of green ZnONPs for structural parameter qualification.**

**Scanning electron microscopy (SEM)**

The surface morphological characterization, composition and grain particle size of synthesized ZnO nanoparticles was determined by Scanning electron microscopy. The garlic synthesized ZnO NPs particle obtained having SEM size (fig.3) ranges from 48.99 nm to 83.71 nm at an average. Remarkably greatest amount of ZnO NPs are identical in dimension together with an insufficient large particle. The lowest value is 48.99 nm with spherical shape with less agglomeration confirm the nanoparticle preparation with SEM size lower than 100 nm same as Jari *et al*.(2022);Islam *et al*. (2024) may had irregular distribution of particle in image due to presence of alkaloids and flavonoids phytochemicals. Here with above references and calibration of that surface area with different production procedure which relies upon growth parameter, concentration of salt, precursor and $P^H$of the mixture solution. Our manipulation in biosynthesis of nanoparticle procedure was pertaining with best result.

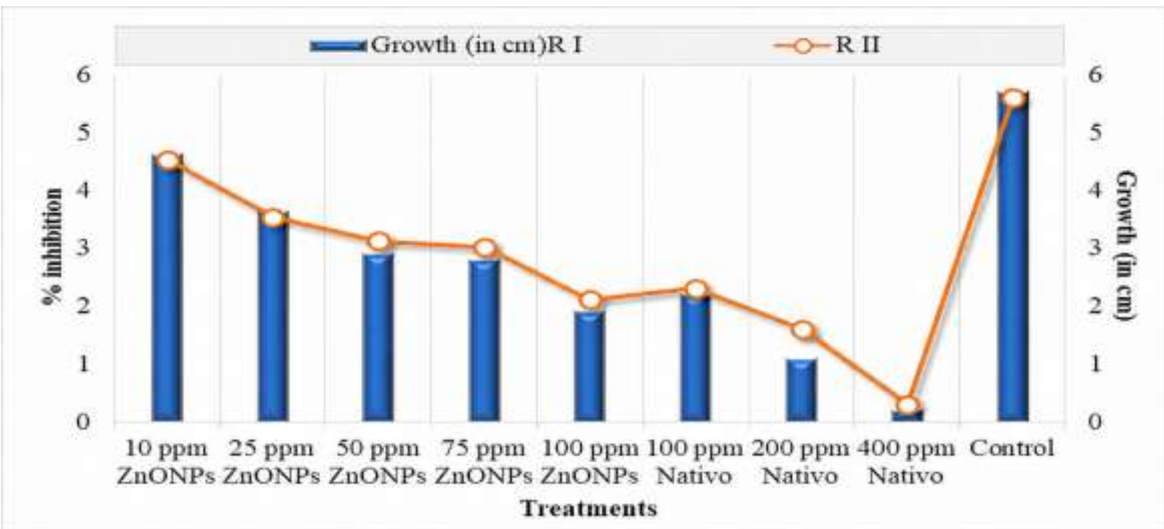
***In vitro*** **analysis of effect of green ZnO NPs on false smut of rice**

As above isolation and purification was done for the false smut of rice pathogen. Then the demonstration regarding effect of respective concentration of ZnONPs against pathogen was tested and observation was summarized below table 1.

**Table 1:- Observation recorded for *in vitro* analysis of effect of green ZnO NPs on false smut of rice**

| Repetition No. | Repetition name | Concentration (ppm) | Growth of pathogen Diameter (Cm.) (Mean of three repetition) | % Inhibition against the control. |
|---|---|---|---|---|
| $T_1$ | ZnO NPs | 10 ppm | 4.60 | 19.29 |
| $T_2$ | Zn O NPs | 25 ppm | 3.60 | 36.84 |
| $T_3$ | Zn O NPs | 50 ppm | 3.07 | 45.61 |
| $T_4$ | Zn O NPs | 75 ppm | 2.87 | 49.12 |
| $T_5$ | Zn O NPs | 100 ppm | 2.06 | 63.85 |
| $T_6$ | Trifloxystrobin 25% + Tebuconazole 50% WG | 100 ppm | 2.30 | 59.64 |
| $T_7$ | Trifloxystrobin 25% + Tebuconazole 50% WG | 200 ppm | 1.17 | 78.94 |
| $T_8$ | Trifloxystrobin 25% + Tebuconazole 50% WG | 400ppm | 0.23 | 95.96 |
| $T_9$ | Control | ---- | 5.70 | 0 |
| | | SE +- | 0.10 | |
| | | CD @5% | 0.29 | |
| | | CV (%) | 5.95 | |

**Graph 01: Graphical representation *in vitro* analysis of effect of green ZnO NPs on false smut of rice with respective treatments**



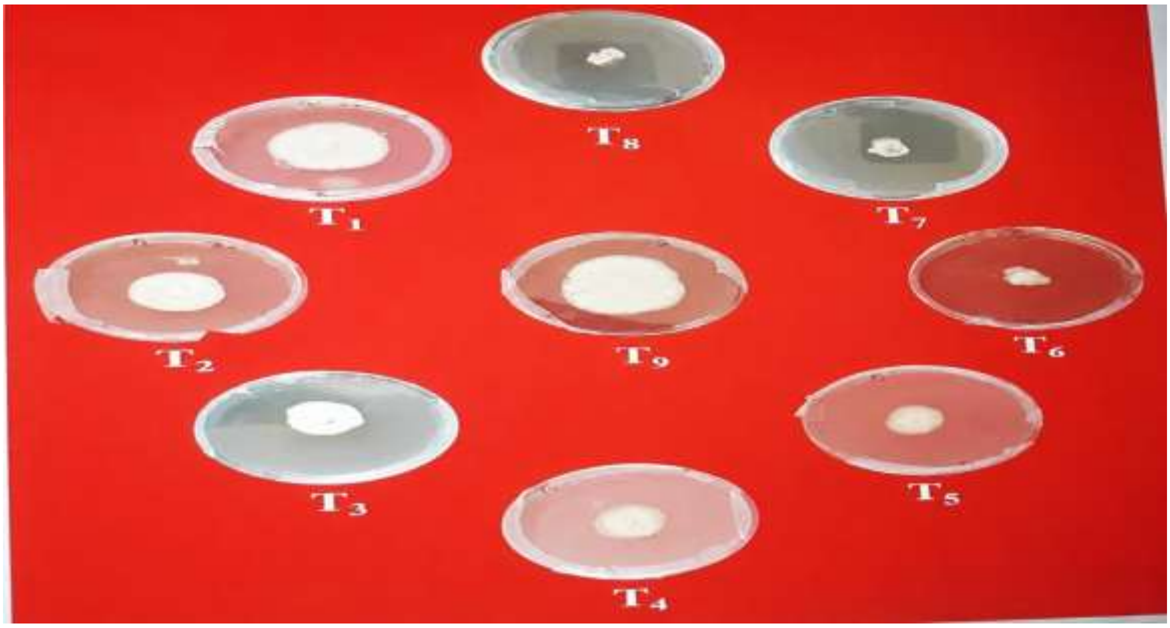



**Figure 5:- Photo showing *in vitro* analysis of effect of green ZnO NPs on false smut of rice with respective treatments**

In study from Photo 1 and 2 showing false smut of rice pathogen myceilal growth with microscopic observation and confirmation of pathogen by its conodiospore. From table 02, graph 1 and fig.5 found significant interaction between the type of ZnONPs and false smut of rice pathogen was observed, with the highest susceptibility. The radial growth of rice false smut fungal pathogen was inhibited ranging from 19.29 per cent to 63.85 per cent in all concentration of green synthesized ZnO NPs. The green ZnO NPs at 100 ppm concentration treatment ($T_5$) inhibit the fungal pathogen growth at 63.85 percent having fungal mycelial growth 2.06 cm with respect of the treatment $T_6$ (Trifloxystrobin 25% + Tebuconazole 50% WG) at same 100 ppm concentration inhibit the fungal pathogen growth at 59.64 percent having fungal mycelial growth 2.30 cm .

The negative chemical control of Trifloxystrobin(25%) + Tebuconazole(50%) WG at 100 ($T_6$),200 ($T_7$) and 400 ppm ($T_8$) concentration leads to inhibition of fungal pathogen with the radial mycelia growth at 2.30,1.17 and 0.23 cm with inhibition per cent at 59.64 percent,78.94 per cent and 95.96 per cent respectively as compared to control treatment with 5.70 cm radial growth of fungal pathogen

The same tune of concentration was associated with previous worker Bhargava *et al*. (2018) and Lipovoskoy *et al*.(2011) and Kalaimurugan *et al*. (2023).Elshafie *et al*.(2023) in antifungal activity of green (moringa leaf extract) and chemically synthesized ZnO NPs against citrus black rot (*Alternaria citri*) found that *in vitro* condition at 2000 µg/ml (2000 ppm) concentration of ZnO NPs inhibit the pathogen at 61 per cent and 52 percent respectively, our garlic ZnONPs were far better as concern with the concentration of ZnO NPs.

The smaller ZnO NPs had better the antifungal activity because of intercellular production of many free radicles like hydroxyl group ,singlet oxygen, super oxide group and nitric oxide radicle they may cross the nuclear membrane of pathogenic cell leads to DNA , chromosome damage and cell death (Shoeb *et al*.2013).

Here that lower concentration of green garlic mediated ZnO NPs was helpful to manage the false smut of rice at non-toxic, eco-friendly and sustainable manner as respect with chemical (Trifloxystrobin 25% + Tebuconazole 50% WG) that were tested against the pathogen *in vitro*. Underline that some quit higher concentration (200 or above) of our green ZnO NPs will be inhibited pathogen at best level Rafiq *et al*.(2022) and Nxumalo *et al*.(2024).Our study had given reliable option against the chemical synthesized nanoparticle and chemical fungicides for management of false smut of rice at *in vitro* condition.

## CONCLUSION

The synthesis of different nanoparticle apart from its physical and chemical method green fabricated methods were simple, smart, non-corrosive, efficient, reliable and effective. The green ZnO NPs had been successfully synthesized using garlic bulb extract as base, reduction compound which acts as stabilizing agent. They reduced the toxicity of Zn O metal oxide particle. The green bio fabricated ZnO NPs were characterized and confirmed by different analytical techniques. The circular to agglomerate shape confirmed by UV-Vis (358 nm), FT-IR range with 400-4000 $cm^{-1}$, and metal oxide nanoparticle formation takes place at less than 500 $cm^{-1)}$ ,SEM (48-89nm at average size ) and XRD analysis reveals four peaks most intense at 13.71 nm with an average size at 8.0 nm with well crystalized hexagonal to wurtzite phase . Under *in vitro* experiment of our green ZnO NPs tested for their antifungal evaluation to inhibits the rice false smut fungal growth at its five different concentration with three negative fungicidal control (Trifloxystrobin 25% + Tebuconazole 50% WG) and one standards control .Our finding shows that green synthesized ZnO NPs manage rice false smut in sustainable level at 100 ppm concentration with 64% inhibition than respective fungicidal concentration. Here we pointed green ZnO NPs use in plant disease management. The green ZnO NPs fungicides provide cost effective and environment friendly alternatives for us became continuous pursuit of sustainable food system. The bio fabrication of Zn O NPs process is an economical method for the synthesis with respect to energy, time and simplicity, cost effectiveness with eco-friendly effect on biosphere.

**Acknowledgment**
We thank to our guide Dr. Patel, Dr. Mehta, Dr.Shulka and Dr. Ware from Department of Plant Protection, NAU, Navsari (Gujarat) for their valuable suggestion, guideline and practical approach with stead fasten encouragement of the topic on earlier drafts and practical laboratory work in preparation of green ZnO NPs and this manuscript.

**Conflicts of Interest**
The authors declare no conflict of interest.

**REFERENCES**
1. Aldalbahi, A.; Seham, A. R.; Ali, A.; Manal A.; Awad, N.S; Fahad S; A. Nasser, A.; Ali B.M.; M. Alateeq, Mohsen, A.,A.,A.,A.;Alkathiri, M.A. and Alrashed ,R.,A.(2020). Green synthesis of zinc oxide nanoparticles, characterizations and multifaceted applications. *Molecules*, Sep; **25**(18): 4198. Published online 2020.
2. Ali, H. and Ali. R. (2023). Eco-friendly synthesis of Zinc Oxide nanoparticles using garlic skin extract: A cost-effective and sustainable approach. *Journal of Xi'an Shiyou University*, Natural Science Edition, vol.**19**.(11).776-781.
3. Alami, Z.Y.; Salem, M.; Gaidi, M. and Elkhamkhami, J. (2015).Effect of Zn concentration on structural and optical proprieties of ZnO thin films deposited by spray pyrolysis. *Adv Energy An International Journal*: AEIJ **2**(4):11–24.
4. Anonymous,(2022).World rice production report by United States Department of Agriculture retrieve from www. worldagriculturalproduction.com
5. Bag,M. K.; Yadav, M.; Banerjee A.and Mukherje, A.K.(2016).Impact of varying planting dates on incidence of false smut disease of rice. Extended Summaries Vol. 2: 4th International Agronomy Congress, Nov. 22–26, 2016, New Delhi, India
6. Bao,J.;Ying,Y.;Xu,Y.;Zhou,X.;Yan,S. and Pang,Y. (2020).Variation in physiochemical properties and nutritional quality in chalky mutants derived from an *indica rice*. *Journal of Cereal Science*, Vol. **91**:102899.
7. Bhargava, P.; Kumar, A.; Kumar, S. and Azad, C.S. (2018).Impact of fungicides and nanoparticles on *Ustilaginoidea virens* causing false smut disease of rice. *Journal of Pharmacognosy and Phytochemistry,* **7**(1):1541-1544.
8. Benshiga, E.; Azhagu, M.S.;Arockia, A. and Brahma N. (2024).*In vitro* antioxidant activity of green-synthesized Zinc Oxide (ZnO) nanoparticles utilizing extracts from *Allium sativum*.*Cureus*,55184/DOI.10.7759.Online.
9. Elshafie H.S.; Osman, A.; Mahmoud, M. E.; Ippolito, C.,I. and Abbas, E. (2023).Antifungal activity of green and chemically synthesized ZnO nanoparticles against *Alternaria citri*, the causal agent citrus black rot. *Plant Pathology Journal,* **39** (3):265-274.
10. Goncagul , G. and Ayaz, E.(2010).Antimicrobial effect of garlic (*Allium sativum*)and traditional medicine. *Journal of Animal and Veterinary Advance* ,**9** (1), 1-4.
**11.** Herrera-Rivera, R.; Olvera ,M.D.L.and Maldonado, A.(2017). Synthesis of ZnO nano powders by the homogeneous precipitation method: use of taguchi's method for analyzing the effect of different variables. *Journal of nanomaterial*, Vol.2017,ID. 4595384.
12. Hassan, H.S.; Elkady, M.F.; El-Sayed, E.M.; .Hamed,A.M.; A.M.Hussein, A.M.and Islam M.M. (2018). Synthesis and characterization of Zinc Oxide nanoparticles using green and chemical synthesis techniques for phenolde contamination. *International Journal of Nano electronics and Materials* ,Vol. 11,No.**2**,179-194.
13. Islam ,M.F.; Islam, S.; Miah, M.A.S.; Huq, A.K.Q.; Saha ,A.K.; Mou, Z.J.; Mondal M.M.H. and Bhuiyan, M.N.I.(2024).Green synthesis of Zinc oxide nanoparticle using *Allium cepa* L.waste peel and its antioxidant and anti-bacterial activity.*Heliyon*,Vol.**10**,Issue.3,e25430.
14. Jari, S.D.; Yasir, S.W. and Razzaq, S.A.(2022).Effect of garlic extract loaded on Zinc oxide nanoparticle against *Sarcocystis in vitro*. *International Journal of Forensic Medicine,* **4**(1):8-12.
15. Karmakar, R.; Das, I.; Dutta , D. and Rakshit ,A.(2016).Potential effect of climate change of soil properties. *Science International* .**4**(2):51-73.
16. Khan, F.; Mohammad, S.; Mohd, A.; Siddiqui, M.A.; Pieter, M. and Ahmad F.(2022).Review green anotechnology: plant-mediated nanoparticle synthesis and application. *Nanomaterial*, MDPI, **12**,673,/nano12040673.
17. Kumar, S. S. and Venkateswarlu, P.(2013).Synthesis, characterization and optical properties of Zinc Oxide nanoparticles. *International of Nano letters,* **3**:1-6.
18. Ladhalakshmi, D.; Laha, G.S.;Singh, R.; Karthikeyan, A.; Mangrauthia, S.K. and Sundaram, R.M.(2012).Isolation and characterization of *Ustilaginoidea virens* and survey of false smut disease of rice in India. *Phytoparasitica* ,**40**:171-176.
19. Laha, G.S.; Prasad, V.; Muthuraman, P.; Srinivas Prasad ,M.; Brajendra A.; Ugandher , T. and Ravindra ,V.B.(2016).Mineral nutrition for the management of rice diseases. *International Journals of Plant Protection*,**9**(1): 310-313.
20. Lu, D.; Yang, X.Q.; Mao, J.H.; Ye, H.L.; Wang, P.; Chen, Y.P.; He, Z.Q. and Chen, F.(2009).Characterizing the pathogenicity diversity of *Ustilaginoidea virens* to hybrid rice in China. *Journal of Plant Pathology,* **91**(2): 443-451
21. Lipovoskoy , A.; Nitize, Y.; Geolanken ,A. and Lubart ,R.(2011).Antifungal activity of ZnO nanoparticles the role of ROS mediated cell injury.*Nanotechnology*,105101,Online.
22. Malik, P.; Shankar, R.; Malik, V.; Sharma, N and Mukherjee, T.K.(2014). Green chemistry based benign routes for nanoparticle synthesis. *J. Nanoparticle,* 1–14.

23. Manu, D.G.; Pramoda S.S.; Ramanathan, A.; Ramchandran, S.; Manonmani, S. and Jayaprakash, (2017).Isolation, characterization and pathogenesis of *Ustilaginoidea viren s* causing disease of rice (*Oryza sativa* L.).*International Journal of Current Microbiology and Applied Science,***6**: 632-640.
24. Masurkar, P.; Singh, R.K.; Bag, M.K.; Singh, H.P. and Hussain, M.E.(2023).Incidence and epidemiology of rice (*Oryza sativa*) false smut caused by *Ustilaginoidea virens* in northern India. *Indian Journal of Agricultural Sciences, 93*(12):1291-1296.
25. Modi, S., and Fulekar, M.H.(2020).Green synthesis of Zinc Oxide Nanoparticles using garlic skin extract and its characterization . *Journal of Nanostructure,***10** (1)20-27.
26. Naseer, M.; ,Aslam ,U.; Khalid, B. and Chen, B.(2020).Green rout to synthesis zinc oxide nanoparticle using leaf extract of *Cassia fistula* and *Melia azedarach* and their antibacterial potential. *Scientific Reports*,Vol.**10**, Article ID 9055.
27. Nxumalo, K.A.; Adeyemi, J.O.; Tshiamo ,B. .; Pfukwa , T.M.; Sunday, N. O. and Fawole, O.A.( 2024).Antifungal properties and molecular docking of ZnO NPs mediated using medicinal plant extracts. *Scientific Reports,* **14**:18071.Retrieved from https://doi.org/10.1038/s41598-024-68979.
28. Parra, M.R. and Haque, F.Z.(2014).Aqueous chemical route synthesis and the effect of calcination temperature on the structural and optical properties of ZnO NPs. *Journal of Materials Science Technology,***3**(4): 363–369.
29. Panse, V.G. and Sukhatme , P.V.(1985).Statistical method for agricultural workers . *Indian Council of Agricultural Research Publication,*, 87-89.
30. Rafiq , H.; Aftab, Z.H.E.; Tehmina , A.; Basharat, A. ; Akram, W.; Uzma Bashir, F. S.; Mirza, M.A.; Muhammad, D. A. and Guihua , L.(2022). Bio-fabrication of Zinc Oxide nanoparticles to rescue mung bean against *Cercospora* leaf spot disease .*Frontiers in plant sciences*, 103389/ fqis.2022.1052984.
31. Ruys, A. (2019). 15-Refractory and other specialist industrial applications of alumina. *Biomed Clin Appl*. Retrieved from https ://doi.org/10.1016/ C2017 -0-01189 -8
32. Schmitz, H. (1930). Poisoned food technique. *Industrial and Engineering Chemistry Analytical Edition,***2**: 361-363.
33. Shoeb, M.; Singh, B.R.; Khan, J.A.; Khan, W.; Singh, B.N.; Singh ,H.B. and Naqvi, A.H.(2013).ROS dependent anti candidal activity of zinc oxide nanoparticles synthesized by using egg albumen as a bio template. *Advance In Natural Science: Nano science Nanotechnology*, **4**:1-11.
34. Solomon ,K.U.; Solomon, T.D. and Gemta, A.B.(2023).Green synthesis method of ZnO nanoparticles using extracts of ginger (*Zingiber officinale)* and garlic bulb (*Allium sativum*) and their synergetic effect for antibacterial activities. *Journal of nanomaterials*, Vol. **2**:1-9.
35. Srivastava, J. P.; Maurya, D.K. and Kushwaha, G.D. (2014).Effect of false smut (*Ustilaginoidea virens*) on seed yield and quality attributes in rice varieties. *Agricultural and Biological Research*. **30**: 196-200.
36. Statista, (2023).Worldwide production of grains in 2022/23, by type (in million MT) http://stastica.com/263977.
37. Strika, I.; Basic, A.; N, Halilovic, N.(2017).Antimicrobial effects of garlic (*Allium sativum L.*).*Bulletin of the Chemists and Technologists of Bosnia and Herzegovina.***20:** 17-20.
38. Tanaka, E.; Ashizawa, T.; Sonada , R.; and Tanaka, C.(2008).*Villosiclava virens* gen .Nov., com. Nov.; teleomorph of *Ustilaginoidea virens* ,the causal agent of rice false smut. *Mycotaxin Research,* **106**: 491-501.
39. Tang, Y.X.; Jin, J.; Hu, D.W.; Young, M.L.; ,Xu, Y. and He, L.P.(2013). Elucidation of the infection process of *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*) in rice spikelet's. *Plant Pathology*,**62**:1-8.
40. Vidya, C.S.; Hiremath, M.; Chandraprabha N.(2013).Green synthesis of ZnO nanoparticles by *Calotropis gigantean. International journal of current engineering and technology,* Vol. **1, (**1):118–120.
41. Vincent, J.M. (1974).Distortion of fungal hyphae in the presence of certain inhibitors. *Nature,* **159**:850.
42. Wang, W.; Fan, M. and Jayakumar, J. M; J (2019). Rice false smut: An increasing threat to grain yield and quality. Intech Open .http://www.intechopen.com/chapter/65860.
43. Yuvakkumar , R.; Suresh, J.; Nathanael, A.J.; Sundrarajan , M. and Hong, S.I. (2014). Novel green synthetic strategy to prepare ZnO nano crystals using rambutan (*Nephelium lappaceum* L.) peel extract and its antibacterial applications. *Materials Science and Engineering*: C, Vol.**41:**17–27.