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Zinc oxide nanorods to degrade phenolics and stored starch of *Cyperus rotundus* tubers management

K. BRINDHA AND [1]C. R. CHINNAMUTHU

Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore – 641003
[1]Department of Agronomy, Agricultural College and Research Institute, Madurai – 625104

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ABSTRACT

The lab experiment was conducted during 2011-12 on purple nutsedge (Cyperus rotundus) to induce the sprouting of buds by degrading the phenolic compounds and exhausting food reserve to reduce the multiplication rate of tubers with Zinc oxide nanoparticles. Zinc oxide nanoparticles (ZnO) were synthesized by wet chemical method characterized with Transmission Electron Microscope, Scanning Electron Microscope, Particles Size Analyzer, Raman Spectrometer and studied their effect on the degradation of phenols, starch, amylose and germination of dormant buds with different concentrations. A standardization experiment was taken up at the preliminary level with different dosages of ZnO in wet and dry conditions to scale down the treatments to a manageable level of thirteen including the control in each of the nanoparticles used. Results revealed that the presence of high amount of phenol (10.5 mg g^{-1}) in the control treatment, no germination (0%) was recorded whereas the ZnO treated tubers at the rate of 1500 mg kg^{-1} registered the lower content of phenol (6.0 mg g^{-1}) and the higher percent of germination (80%), longer root, shoot length (20.43 cm, 29.00 cm) and higher vigour index (3954) in dry method. In case of wet method, higher amylose content of 52.0 mg g^{-1}, lower content of starch (108.0 mg g^{-1}) , phenol(4.2 mg g^{-1}) with 80 per cent germination, longer root, shoot length (20.00 cm, 29.00 cm) and higher vigour index (3920) was noticed with the concentration of 2250 mg kg^{-1}. The untreated tubers (control treatment) recorded lower amylose content (10.0 mg g^{-1}) with higher phenol (9.9 mg g^{-1}) and starch content of 148.0 mg g^{-1} with 20 per cent germination.

Keywords : Biochemical components, purple nutsedge tuber, zinc oxide nanoparticles

The demand for food crops is increasing day by day and we are in a situation to feed the growing population. However the plants out of place growing along with crop plants known as weeds became one of the major threats to limit the yields. Suitably managing this limiting factor will certainly accomplish the production and productivity of crops and preserve the natural resources. Charles (1997) reported that the purple nutsedge is considered as one of the worst weeds of the world widely distributed throughout the tropics and subtropics in 52 different crops and in 92 countries. It ravages the cultivated fields very quickly and causes one hundred per cent yield loss at times. Bryson *et al*. (2003) reported that the purple nutsedge is highly competitive and causes yield reductions of various crops ranging from 23 to 89 per cent and particularly 62 to 85 per cent reduction of seed cotton yield compared with no purple nutsedge infestation. Management of this weed will be one of the major tasks because of its perennial nature. Many cultural and chemical methods were tried to manage *Cyperus* spp., however the results are not encouraging. Accumulation of phenolic compounds during the stress condition prevents the germination of tubers to tide over the unfavourable condition. Further the poor translocation of foliar applied herbicides kill only the primary tubers leaving the others in chain unaffected lead to enlarging of weed seed propagules reserve as season advances. Therefore the nutsedge management strategies must include a long-term commitment to prevent the new tuber formation, breaking the dormancy and killing the viable tubers. In order to have a better insight into the work done already on biology, competitive nature, control measure taken in the recent past and the possibilities with the new science, the nanotechnological applications to manage this perennial sedge weed. Hence, the present study was carried out to evaluate the zinc oxide nanoparticles mediated breaking of dormancy and exhausting the food reserve in the tubers of *Cyperus rotundus*.

A laboratory study was conducted at the Department of Nano Science and Technology, Tamil Nadu Agricultural University during 2011-12. Tubers of purple nutsedge (*Cyperus rotundus*) collected in bulk from the garden lands of Velampatti (11°9’29"N 78°21’54"E), Trichy district, Tamil Nadu during 2011-2012 formed the base material for the study. The Zinc oxide (ZnO) nanoparticles were synthesized in the laboratory through chemical routes. For which 0.45 M aqueous solution of zinc nitrate Zn $(NO_3)_2.4H_2O$ and 0.9 M aqueous solution of sodium hydroxide (NaOH) were prepared in distilled water. Then, the beaker containing NaOH solution was heated to the temperature of 55 ºC. The Zn $(NO_3)_2$ solutions were added drop wise (slowly for 40 min) to the above heated solution under high speed stirring. The beaker was sealed at this condition for two hour. The precipitated ZnO nanoparticles were cleaned with deionized water and ethanol then dried in atmospheric air at about 60 ºC.

Short communication
Email: bindiya14@gmail.com

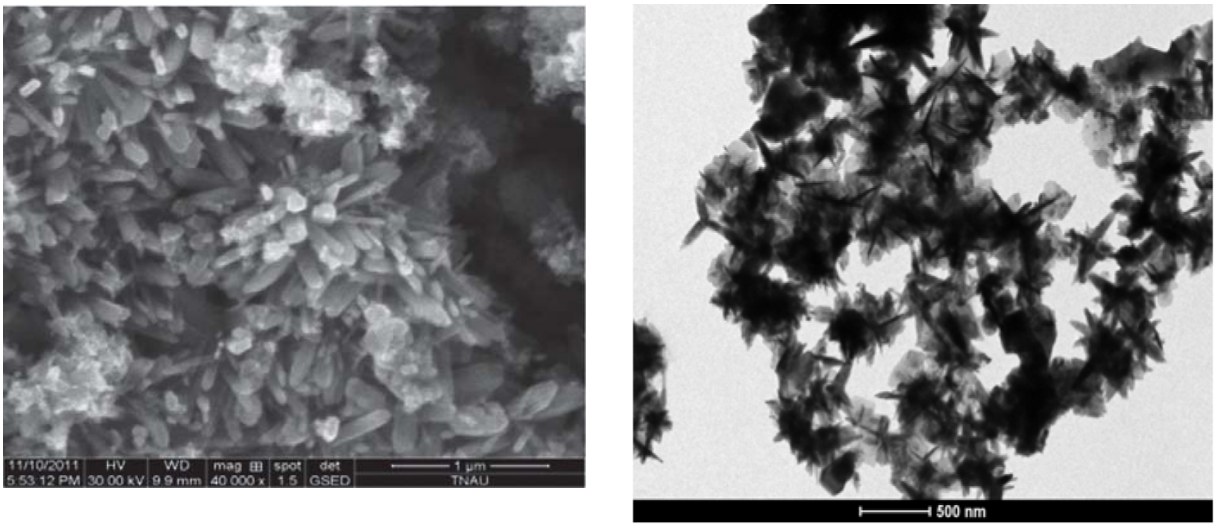

Fig. 1 : Micrograph of Zno nanoparticles a) SEM b)TEM

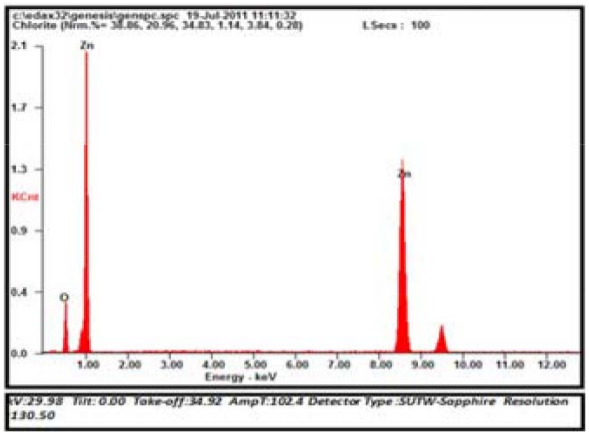

Fig. 2 : EDAX studies of ZnO nanoparticles

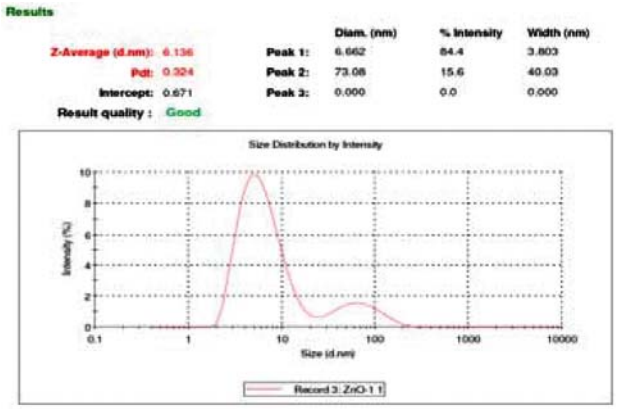

Fig. 3 : Particle analysis of ZnO nanoparticles

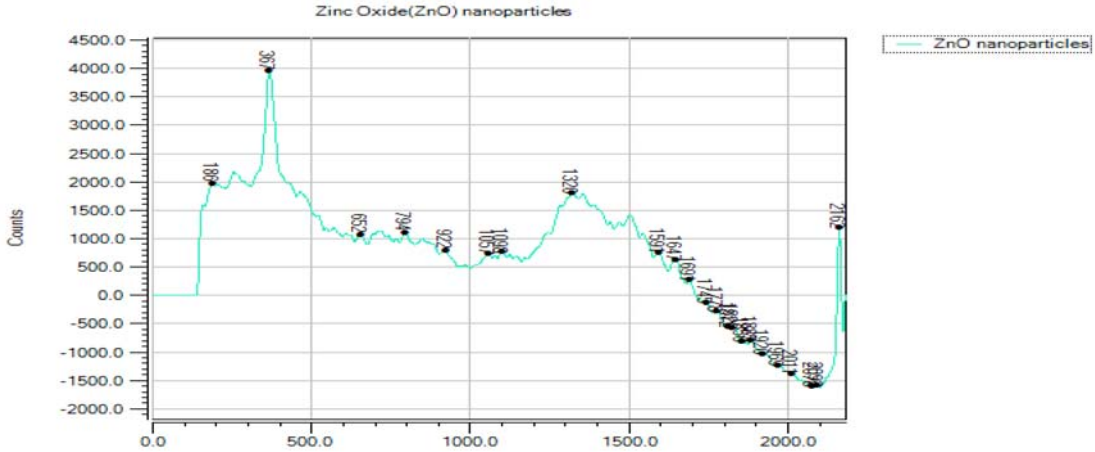

Fig. 4 : Ramanspectroscopy of ZnO nanoparticles

Table 1: ZnO nanoparticles on the biochemical composition and germination , root, shoot length and vigour index of *Cyperus rotundus*

ZnO nanoparticles concentration (mg kg^{-1})	Starch (mg g^{-1})	Amylose (mg g^{-1})	Phenols (mg g^{-1})	*Germination (%)	Root length (cm)	Shoot length (cm)	Vigor index
Dry method							
T_1- 0(Control)	142	8	10.5	0 (0.28)	0.00	0.00	0
T_2-250	137	10	9.4	20 (26.85)	9.10	15.30	488
T_3-500	133	13	9.1	33 (35.46)	7.70	12.30	660
T_4-750	131	15	8.9	40 (39.69)	10.90	18.10	1160
T_5-1000	128	18	8.6	40 (39.69)	10.00	7.00	680
T_6-1250	125	21	8.2	40 (39.69)	11.16	16.16	1093
T_7-1500	102	44	6.0	80 (64.60)	20.43	29.00	3954
T_8-1750	107	40	6.4	73 (59.64)	12.50	16.30	2102
T_9-2000	110	37	6.7	60 (51.47)	15.73	12.90	1717
T_{10}-2250	112	32	7.0	60 (51.47)	7.16	7.83	900
T_{11}-2500	115	30	7.4	40 (39.69)	5.50	11.40	676
T_{12}-2750	119	27	7.7	40 (39.69)	8.33	13.43	870
T_{13}-3000	122	24	7.9	40 (39.69)	8.06	17.50	1022
SEd	**1.99**	**0.44**	**0.13**	**0.49**	**0.17**	**0.24**	**24.70**
LSD (0.05)	**4.10**	**0.90**	**0.27**	**1.01**	**0.36**	**0.50**	**50.77**
Wet method							
T_1- 0(Control)	148	10	9.9	20 (26.56)	3.20	3.50	402
T_2-250	130	22	7.9	40 (39.23)	5.50	11.40	676
T_3-500	113	45	5.3	47 (43.28)	8.06	17.50	1201
T_4-750	133	19	8.3	33 (35.06)	7.16	7.83	495
T_5-1000	114	41	5.8	47 (43.28)	8.33	13.43	1023
T_6-1250	110	49	4.9	73 (58.69)	10.90	18.10	1740
T_7-1500	125	30	7.0	40 (39.23)	11.16	16.16	1093
T_8-1750	118	37	6.2	47 (43.28)	12.50	16.30	1354
T_9-2000	121	33	6.6	40 (39.23)	9.10	15.30	976
T_{10}-2250	108	52	4.2	80 (63.43)	20.00	29.00	3920
T_{11}-2500	128	25	7.5	40 (39.23)	15.73	12.90	1145
T_{12}-2750	141	12	9.5	27 (31.30)	10.00	7.00	459
T_{13}-3000	138	25	8.8	33 (35.06)	7.00	12.30	637
SEd	**2.05**	**0.54**	**0.11**	**0.47**	**0.17**	**0.24**	**23.81**
LSD (0.05)	**4.22**	**1.12**	**0.24**	**0.98**	**0.36**	**0.50**	**48.95**

** Figures in the parentheses are the transformed values*

Morphology of the sample was investigated using Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM), Particle Size Analyzer and Raman Spectroscopy.

Different concentration of Zinc oxide nanoparticles (250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750 and 3000 mg kg^{-1}) were used both in wet (liquid from) and dry (powder form) methods. In case of wet method, ZnO nanoparticles were dispersed in distilled water by sonicating for 5 min separately. The purple nutsedge tubers were soaked in that solution for 3 days. The soaked tubers were removed and dried again to the original moisture content. The purple nutsedge tubers soaked in water served as control. For dry method, tubers were dressed with ZnO nanoparticles as mentioned above concentrations in screw capped glass bottles at room temperature separately. The glass bottles containing tubers with nanoparticles were shaken gently for 3 min for 5 times at an interval of 3 hours. Tubers shaken without nanoparticles served as control. Their effect on the degradation of phenol, starch, and germination of dormant buds with different concentration and wet (liquid form) and dry (powder form) methods and the free radical scavenging activity of ZnO nanoparticles were analyzed by DPPH Assay as per the method described by Braca *et al.* (2001).

ZnO nanoparticles were synthesized and characterized with Particle Size Analyzer for estimating the average particles size and size distribution pattern [diameter of 199.2 nm and the width of 65.36 nm (Fig.3)]. Scanning Electron Microscope to determine the surface morphology (Fig.1a) with the shape of bunches of flowers. Each bunch is gathered of closely packed nanometer scale rods and forms radiating structures. The EDAX image of ZnO showed that, on weight basis, it contains 82.69 percent Zn and 17.31 percent Oxygen in the K shell and an atomic percentage of 53.90 and 46.10 are Zn and Oxygen, respectively (Fig.2). ZnO nanoparticles were scanned using TEM to determine the internal structure (Fig.1b) and showed the rod shaped nanoparticles with approximately 16 nm diameter. The Raman spectrum was studied for ZnO nanoparticles. The relative intensities of the peaks were used to know the information on the composition of a mixture. It is more visible (Fig. 4) that the intensity of peaks for ZnO was 367 cm^{-1} and the same way the reference peaks were confirmed the sample contains ZnO nanoparticles.

Results of the study further revealed that effect of ZnO nanoparticles on the biochemical components, germination percentage, root, shoot length and vigor index in wet, dry methods presented in table 1. It is evident that the presence of higher amount of phenol (10.5 mg g^{-1}) in the control treatment and no germination (0%) was recorded. Whereas, the ZnO treated tubers at the rate of 1500 mg kg^{-1} registered lower content of phenol (6.0 mg g^{-1}) and higher percent of germination (80%), longer root, shoot length (20.43, 29.00 cm) and higher vigour index (3954) in dry method. With respect to starch content of tubers, the ZnO nanoparticles significantly reduced the starch content. The reduction of starch was noticed upto 1500 mg kg^{-1} concentration, there after the trend was not sustained. The same trend for the other parameters like amylose and phenols were also observed. In case of wet method, higher amylose content of 52.0 mg g^{-1}, lower content of starch (108.0 mg g^{-1}) and phenol (4.2 mg g^{-1}) with 80 per cent germination, longer root, shoot length (20.00, 29.00 cm) and higher vigour index (3920) was noticed with the concentration of 2250 mg kg^{-1}. The untreated tubers (control treatment) recorded lower amylose content (10.0 mg g^{-1}) with higher phenol (9.9 mg g^{-1}) and starch content of 148.0 mg g^{-1} with 20 per cent germination. ZnO nanoparticles act as a power house of electron donor possessing ability to degrade organic and inorganic compounds (Poly phenols) present in the tubers. The reduction of starch and phenols may be attributed due to the higher reaction property of ZnO nanoparticles (Shah and Belozerova, 2008). However, after certain concentration the growth of root and shoot was found to decline. This may be due to the fact that at lower concentration, the nanoparticles act as growth promoting substance and higher concentration act as growth retarding one. ZnO nanoparticles increased the level of IAA in the roots (sprouts), which in turn increased the growth rate of plants. Further the ZnO nanoparticles are having more surface area due to their small size (Shah and Belozerova, 2008). It acts as a peroxidase enzyme; it will donate electrons and reduces the phenol content of the tubers, responsible for the dormancy, allow the tubers to germinate. This may be due to the reason that the reduction in the phenol content increased the activity of alpha-amylase present in the tubers. The hydrolytic enzyme alpha-amylase catalyzed the breakdown of polysaccharides into glucose; an energy source for plant growth induced the sprouting of bud and germination. The antioxidant activity of ZnO nanoparticles was investigated in the present study (Braca *et al*. 2001). It was observed 81 percent antioxidant activity in ZnO nanoparticles.

Among the two methods, dry method was quicker (1500 mg kg^{-1}) than the wet method (2250 mg kg^{-1}) to break the dormancy of purple nutsedge tuber and enhanced the germination. At this juncture, the control of nutsedge tubers could be achieved by using any of the available herbicides.

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