

Physicocytological Invigoration of Root Meristematic Chromosomal Anomalies of *Celosia argentia* L. by the Genetic Potential Stress Influence of Water Hyacinth Deproteinized Foliage Whey

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In earlier studies, high concentration of DPJ from various forages inhibited germination, induced mutations in some seedlings and caused phytotoxic phenotypical changes in plants during growth. The physiological changes were absence of leaves and presence of more stipules in few pea plants. DPJ obtained from Lucerne and *Eicchornia crassipes* found causing chromosomal aberrations at high concentrations i.e at 0.50%, 1%, 1.5% and 2% in onion and garlic root meristems during the process of mitosis. It showed normal growth of *Celosia* plants by the effect of *Eicchornia* DPJ, when the concentrations are increased from 0.1 to 1 % level. The objective of research is to determine DPJ as mutagenic agent for effecting the cell chromosomal changes for the purpose to optimize the crops physiologically and genetically by its stress application.

During present investigation, the so called antipathogen water hyacinth DPJ potentiality changed the chromosomal response of *Celosia* at 1.5 %, and 2 % high concentrations. It was compared with the control. *Celosia* consists of the anomalous secondary growth in the root or stem. In mitotic study, mostly restitution prophases and unorientation of anaphases were found in the germinated seedling somatic cells because of the exogenous application of DPJ to the seeds.

Key words: Germination, growth, root meristem, phenols, amino acids, stress, mutation, restitution prophases, phenotypic, chromosomes

Cockscomb (*Celosia argentea* var *cristata* L.), an almost sterile hybrid between them, and eight F₂ plants raised from seed from the hybrid have been studied cytologically. The hybrid possessed a chromosome number ($2n=54$) intermediate between those of the parents ($2n=36$) and 72 for cockscomb and *C. argentea*, respectively (William, 1954). In previous investigation, influence of DPJ was studied on leguminous plants pea and cowpea growth. DPJ enhanced the growth and the protein content of the plants. DPJ also induced the seed germination of maize, ragi and horsegram (Jadhav, 2018). After the treatment of lethal dose of DPJ of lucerne as a fertiliser when given to pea plants, there were phenotypic changes found like presence of more stipules and tallness of the stems (Jadhav, 2009a). In effect of DPJ on root growth, it was found that it induced more number of onion roots and nodulation in cowpea plants (Jadhav, 2017). At high concentration effect of DPJ in *Allium cepa*, there were observations of the chromosomal aberrations like micronuclei, anaphasic bridges, unorientation in metaphases, unequal daughter nuclei in telophases (Jadhav and Mungikar, 1998; Jadhav, 2009b). When the lethal dose of DPJ induced phytotoxicity in pea plants, it caused anomalous tall length of the plants with absence of leaves and only presence of stipules in some plants. DPJ contains flavonoids, phenolic compounds, lycopene etc. (Oboh, 2016). Chowdhary *et al.* (2002) worked on the growth of actinomycetes on DPJ, considering it as a medium.

In earlier studies, DPJ showed its potential in reducing the sizes of stomata to prevent excess transpiration with reference to drought prone area plants. DPJ consists of the potentiality to optimize the process of nitrogen fixation as because of its application to the soil if is exploited as manure (Jadhav *et al.*, 2018; Jadhav and Gare, 2018)

A new allopolyploid species of *Celosia*, namely, *C. whiteii*, collected in Malaya and originating from a natural cross between *C. argentea* L. ($2n=72$) and *C. cristata* L. ($2n=36$), has been described and the somatic chromosome number determined as $2n=108$. A somatic chromosome number of $2n=18$ has been determined for another species of *Celosia*, namely, *C. trigyna* L. The basic chromosome number for the genus has been

determined as nine. Of the four species of *Celosia* so far studied cytologically, each represents a different level of ploidy, from diploid ($2x$) to dodecaploid $12x$ (Grant, 1961).

The present determination is the exploitation of DPJ form *Eicchornia crassipes* L. Cornelius *et. al.* (2016) showed a good antioxidant activity of *Eicchornia crassipes* extracts, particularly for dichloromethane and ethyl acetate, thus as an agent supplier of antioxidants. Moreover, it can be used as an alternative to help the control of *E. crassipes* blooms, which can affect the aquatic ecosystem. Phytochemical studies have reported compound as phenols, steroids, phenalene and humic acid commonly in plant that absorbs metals from the environment. It is antimicrobial for *E. coli*, *Candida albicans*. *E. crassipes* forage was found to be rich in flavonoids, amino acids, crude protein, cyanide, phosphate, silicon, calcium, potassium, organic matter and carbon. It grows in almost neutral pH, substantial concentration of dissolved oxygen, an increased rate of biological oxygen demand, a substantial concentration of nitrates and very low salinity and alkalinity. Investigation of various amino acids in *Eicchornia* leaf extract indicates that it consists of phytohormones, as tryptophan and methionine are precursors of auxins, gibberellins and cytokinins (Lara-Serrano *et al.*, 2016; Nyananyo *et al.*, 2007). P. Jayanthi *et al.* (2011) investigated the proteins in *Eicchornia* leaves by ninhydrin and biurets method and phenols. The presence of phenolic content indicates the presence of salicylic acid, a plant hormone which is a compound of phenol in *Eicchornia*, favoured the seed germination and seedling growth of *Celosia* at appropriate concentrations of DPJ.

In order to evaluate the potential of DPJ as a mutagen to optimize the crop characteristics phenotypically and genotypically its effect on mitosis in root meristem is the present objective (Kothekar *et al.*, 1995; Satpute and Ghagre, 2009; Esra *et al.*, 2018). *Celosia* seeds were germinated by the treatment of increasing concentrations of water hyacinth DPJ. The results obtained were increase in the length and weight of the seedlings as per the increase in DPJ concentration (Jadhav, 2018). During present

investigation, the DPJ dose application to seeds exogenously was given at high concentration beyond 1 to 2% to evaluate anomalies.

MATERIALS AND METHODS

Preparation of Eicchornia DPJ

By the process of green crop fractionation, the forage of water hyacinth was pulped and squeezed to obtain the juice. The juice is heated to 90°C, filtered in order to obtain the leaf protein concentrate (LPC) on filter paper. The curd like extract after heating forms the supernatant is called as deproteinized juice (DPJ) or leafy whey, as the proteins gets coagulated by separation. The DPJ is dried in oven and various concentrations were prepared by weighing it in grams to treat for the seed germination of *Celosia* by paper towel method. High concentrations of DPJ were 1, 1.5 to 2 %. The seeds were soaked for 24 hours in DPJ made up of *Eicchornia crasipes L.* to have the effect on germination of seeds. The cytological studies were followed by using the root tips of *Celosia argenticia* seedlings after the germination.

Cytological Investigations of Celosia Roots Grown In Vitro

Ninety (90) seeds of *Celosia argenticia L.* (Century mixed) were germinated on moist filter paper. Primary roots produced *in vitro* were also made into permanent slides and analysed for cellular behaviours, such as Mitotic index and chromosome count. The experiments were conducted using a light microscope images. Treated and Control seeds were placed in folded whatman paper, lined with moist tissue paper. The healthy root tips 1 to 1.5 cm were cut which were pre-treated with 1 %, 1.5 % and 2 % of DPJ. The root meristems were then fixed in 1:3 (v/v) glacial acetic acid and alcohol solution for 24 hours to fix cell division. These isolated meristems were preserved in 70 % alcohol until ready for viewing. The meristems were hydrolysed in 1N HCl (hydrochloric acid) for 15 minutes and squashed on a glass slide. They were then stained in 2 % acetocarmine for 1 hour and viewed under light microscope. Frequency of each phases of cell division was counted and the Mitotic index (MI) estimated. Five replicate slides were prepared for each treatment (Abubakar et al, 2015).

RESULTS AND DISCUSSION

During the amino acid analysis in the *Celosia* foliage itself, it revealed high contents of the essential amino acids with methionine as the limiting amino acid. Presence of methionine in extract reveals its hormonal availability (Aodele and Olajide, 2011). In earlier experiment, there was no influence of DPJ at low concentration i.e at 0.12 % during the seedling growth of *Celosia*. When the concentrations increased at 0.50 %, 0.75 % and 1.00 %, there was found promotion in length and weight of the seedlings of *Celosia* plants as per the enhancing concentrations of DPJ by soaking treatment. Table 1 illustrates that comparatively during cell division, majority of abnormalities were found in the anaphases by unequal segregation or unorientation of the chromosomes. Anaphases were also had bridges and laggards. There was incomplete arrest of cell division at the stage of prophase because there was restitution in it at 15.21 % of the mitotic index. Many of the chromosomes were having the stickiness. Majority of prophases were suffered from scattering, disintegration, fragmentation and granulation. Balloon nuclei were found in case of metaphases and during the stage of telophase. The telophases were less in number. Comparatively the telophases were very small in sizes. The process of cell division was declined after the stage of anaphase, because of impact of higher concentration of DPJ. Therefore the table indicates that there was no complete mitotic arrest because of the application of DPJ at 1.5 % level. DPJ when treated at 2% level, there was arrest of mitosis as prophases suffered from aberrations like scattering and condensation in many nuclei. Hence the total chromosomal aberrations by calculating mitotic index because of DPJ was 93.45 %. In earlier findings it was already investigated that high concentration of lucerne DPJ i.e at 2% level, caused mitotic arrest in many nuclei. Jadhav and mestry (2018) founded that *Allium cepa* DPJ inhibits the enzyme protease of the fungi *Trichoderma viride*. Haggag *et al.* (2017) found antifungal activity of *Eicchornia* against six pathogenic fungi viz. *Aspergillus flavus*, *A. niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Candida albicans*, and *Fusarium solani*. Therefore, very few normal nuclei were found by *Eicchornia* DPJ

influence. However the investigation proves that there was the mutagenic influence of DPJ on seedlings of *Celosia* plants. In order to study the change in chromosomal number of *Celosia* or other genus because of DPJ, research is advisable. DPJ influence

varies as per the species of plants used. The study prevails that the relevant concentration of DPJ in the present investigation for the purpose of mutational studies was of 1.5 % for giving the treatment.

Table 1. Mutagenic influence of *Eichhornia* DPJ on seed germination at various concentrations and study of chromosomal aberrations in the somatic cell nuclei of *Celosia argentea.L* from root meristems.

No.	DPJ Treatment (%)	Chromosomal abnormalities (CA)	Mitotic Index (%)
1	2	Restitution of prophases	15.21
2	2	Scattering or disintegration of prophases	8.69
3	2	Fragments or granulated nuclei	13.04
4	2	Condensation of the nuclei	8.69
5	1.5	Baloon nuclei of metaphases	6.52
6	1.5	Stickiness in chromosomes	17.39
7	1.5	Unorientation or unequal segregation	23.91
		Total (M.I.) Percent of abnormalities	93.45

Table 2. Percent Mitotic Index of each stages by DPJ influence on *Celosia* somatic cells.

No.	Chromosomal Abberations in mitotic stages	Mitotic Index, (%)
1	Prophase	50
2	Metaphase	39.13
3	Anaphase	26.08
4	Telophase	15.21
5	Total	67.39

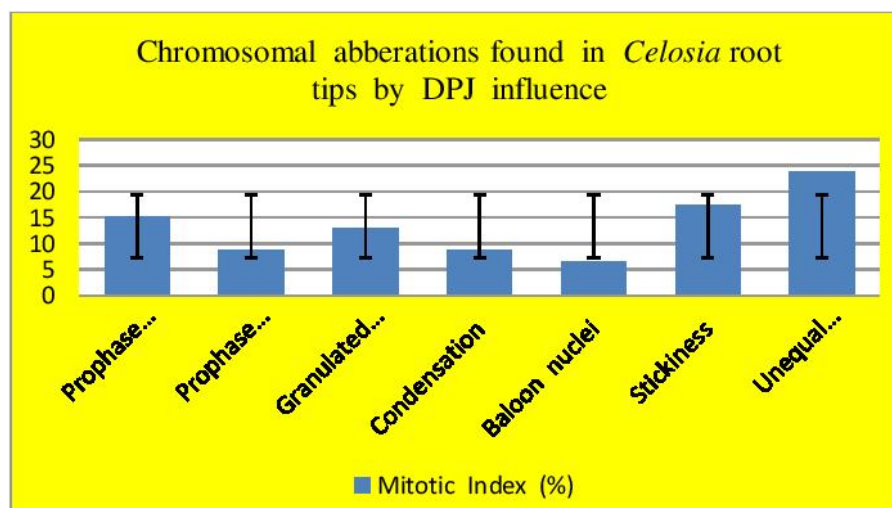


Figure 1. Graphical presentation of the chromosomal aberrations found by the influence of the DPJ on somatic cell division of *Celosia argentea*.

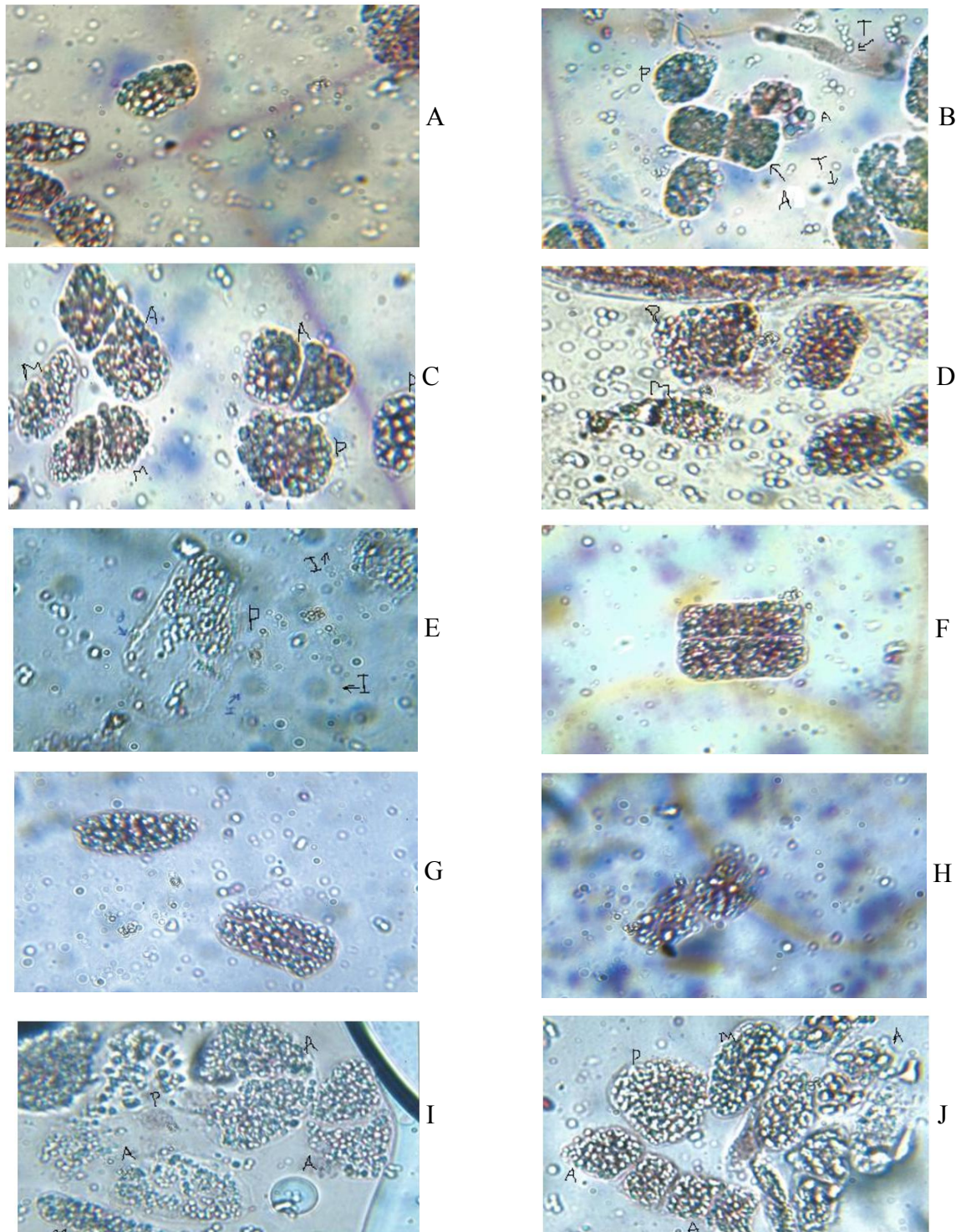


Figure 2. Illustration of the somatic chromosomal anomalies (CA) of *Celosia* root tips. (A) Stickiness of the chromosomes, Normal Prophase and metaphase. (B) Telophase having long distance among the daughter cells, prophase and disturbed anaphases. Normal telophase having small daughter nuclei. (C) Disturbed metaphase and unequal segregation of anaphases. (D) Restitution of prophase and unorientation of balloon shaped metaphase. (E) Interphases and restitution of prophase. (F) Sticking of two metaphases together. (G) Late and earlier metaphases. (H) Disturbed Anaphase with bridges and laggards. (I) Disintegrated and scattered prophase, unequal segregation in anaphases and presence of metaphase. (J) Unequal segregation in anaphases, restitution, condensation and fragmentation of prophases and earlier metaphase. Labelled figures (pictures) P=prophase, M=Metaphase, A=Anaphase, T= teophase.

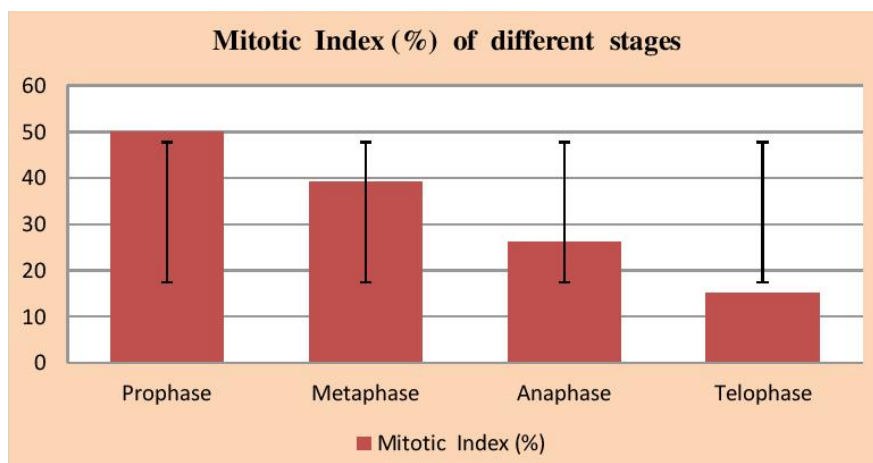


Figure 3. Graphical illustration of chromosomal anomalies in different mitotic stages.

Graphical presentation in figure 1 illustrates the higher abnormalities scores of chromosomes were found during the anaphase stage i.e of unequal segregation of chromosomes towards two opposite poles. Figures 2 (A) to (J) indicates that there were chromosomal aberrations. The nuclei found of cockscomb somatic cells were broader in size comparatively with *Allium cepa* and *A. sativum*. During slide preparation, due to excessive chromosomes, there was breakage and dispersal of many chromosomes found on the slide.

Figure 2 (E) very clearly reveals presence of normal interphases. Normal prophases are shown in figures 2 (A) and (C), metaphases in figures 2 (A), (G) and (I). Normal anaphase and telophase shown in figure 2 (B).

The restitution and disintegration of the prophase stages are depicted in figures 2 (D), (E) and (I). Abnormal metaphases are shown in figures 2 (C), (D) and (F). There are two normal prophases and one normal anaphase in figure 2 (B). Abnormalities in anaphase was found dual. Those laggards and unorientations among chromosomes. Unequal segregation in anaphase is shown in figures 2 (C), (I) and (J). Figure (H) shows the anaphasic bridges and laggards. In figure 2 (B), the telophase is consisting of long abnormal distance among the two daughter nuclei.

Mitotic activities observed in the cells of the treated plants and the control are presented in Table 2. Estimated mitotic index values showed that DPJ concentration at 2% plants which had the highest percentage of abnormalities illustrated in table 1. Mitotic

index values obtained illustrated in table 2 determines that in all the untreated cells of the control was 80.36, higher than that of the total of treated. Treatements total had the least value (67.39). The most occurring mitotic stages is the prophase (50) followed by the metaphase (39.13) and anaphase (26.08). Least stages of telophases were found as these stages were not got divided from anaphase (Figure 3). Reason of least telophases is unequal segregations of chromosomes and bridges i. e the biphasic anomalies in anaphasic stages. There was decreased mitotic index of the treated plants compared with the control. It was reported that there were inhibitory effects of water hyacinth DPJ on the mitotic index of *Celosia*. This means that DPJ can have a genotoxic and mutagenic effects. *C. argentea*, the plant in which aberrations induced, contains saponins, cyclic-peptides, phenols, fatty acids, amino acids, minerals, of which saponins are the main pharmacological active agents and might be the promising target for further studies due to their bioactivities and the mechanisms of saponins have been performed. Anti-mitosis in the moroidin (6)/celogentin families in *Semen Celosiae* has been reported (Tang *et al.*, 2016).

Similar effect of DPJ from lucerne was observed on onion and garlic root tips. It is opined that ATP deficiency caused by excessive constituents of DPJ which comes during heating of leaf juice at 90°C after filtration. It may be one of the reasons for the decrease in the mitotic index and demands for ATP of dividing cells are much higher as compared to non proliferating

cells. DPJ is enriched with all nutrients especially salts, hormones, DNA and RNA. DPJ also contains non protein nitrogen and very few amino acids despite it is deproteinised. These Chromosomal aberrations were analyzed in metaphase chromosomes detected within the cell population of only a single primary root out of the whole seedling grown by DPJ treated seeds after germination, which is a strong evidence for its spontaneous nature (Nikolova, 2015).

CONCLUSION

The present study invigorated that, the high concentration of *Eichhornia* DPJ acted as the mutagenic agent on *Celosia argentea* weed/ornamental plants by disturbing the cell cycle. However, study reveals that the mutagen DPJ have the potentiality to induce the complex chromosomal changes in *Celosia* plants at 1.5% as this plant bear the anomalous secondary growth of stem. The stress of DPJ by its application have the efficacy in physiological changes and the nature of chromosomes in plants because of its chemistry and hormonal content. Investigation of change in chromosomal number in plants due to DPJ will be the next objective of the research. Other commercial seeds will be taken into consideration for genetical research to make the crops more optimistic like production of disease resistant variety. Chromosomal aberrations play a vital role in evolution as they generate variation in a natural population. Aberrations result in altered linkage relationships and this has been exploited for breeding experiments.

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REFERENCES

Abubakar A, Falusi, A. O, Daudu O. A. Y, Oluwajobi A. O, Dangana, M. C, Abejide D. R. (2015) Mutagenic Effects of Sodium Azide and Fast Neutron Irradiation on the Cytologica Parameters of M₂ Lagos Spinach (*Celosia argentea*

var cristata L.). *World Journal of Agricultural Research*. **3**, 107-112.

Ayodele J. T. and Olajide O. S. (2011) Proximate and Amino Acid Composition of *Celosia argentea* Leaves. *Nigerian Journal of Basic and Applied Science* (2011), **19** (1), 162-165

Chowdhary N.B., Naik V.N., Sharma D.D. and Govindaiah (2002) Deproteinised Mulberry leaf juice: The new media for growth of microorganisms, *International Journal of Industrial Entomology*. **5** (2), 217- 220.

Cornelius M.T.F., Chapla V., Braun G., Sarragiotto M.H., Schirmann J. and Olguin C.F.A. (2016) Phytochemical and biological investigations of *Eichhornia crassipes* (Mart.) Solms. *Journal of Chemical and Pharmaceutical Research*, **8** (3), 564-570

Desai S. R. (1971) Morphological and Cytological Studies on Amaranthaceae. Bombay College of Pharmacy, Bombay, India, 349 - 353

Esraa, M. El-Azab, Mohamed Soliman, Elham Soliman and Abdelfattah Badr (2018) Cytogenetic Impact of Gamma Irradiation and Its Effects on Growth and Yield of three soybean Cultivars. *Egyptian Journal of Botany*. **58**, (3), 411 – 422.

Grant W.F. (1954) A cytological study of *Celosia argentea*, *C. argentea* Var. *cristata* and their hybrids. *Botanical Gazette*, **115** (4), 323–336.

Grant W.F. (1961) Speciation and basic chromosome number in the genus *Celosia*. *Canadian Journal of Botany*, **39** (1), 45-50

Haggag, M. W., Abou el ella, S. M. and Abouziena, H. F. (2017) Phytochemical analysis, antifungal, antimicrobial activities and Application of *Eichhornia crassipes* against some plant pathogens. *Planta Daninha* ; v35 : e017159560 Doi: 10.1590/S0100- 83582017350100026

Jadhav R.K .(2009 b) Effect Of Deproteinised Juice From *Eicchornia crassipes*. L On Mitotic Cell Division of *Allium cepa* L. and *Allium sativum*. L. *Bioinfolet*, **6** (3), 245 - 248

Jadhav R.K (2009 a) Phytotoxic Effect Of Deproteinised Leaf Juice (DPJ) of Lucerne (*Medicago sativa* L)

- On Pea (*Pisum sativum*. L) *Bioinfolet*, **6** (3), 240 – 241.
- Jadhav R.K (2015) Distribution of Ash, Calcium and Phosphorus in the Byproducts Of Green Crop Fractionation. *International Journal of Bioassays* **4.9**, 4329 - 4332.
- Jadhav R.K (2017) Effect of deproteinised Juice (DPJ) on Root Growth and Nodulation of Leguminous Plants. *Journal Bionanofrontier* **10** (1), 84 – 87
- Jadhav R.K (2018) Response of seed germination and seedling growth by the deproteinised juice (DPJ) from various forages. *Biochemical and Cellular Archives*, **18** (1), 1181-1188.
- Jadhav R.K and Chhaya G. (2018) Influence of deproteinised foliage fluid liquid biofertilizer on nitrate reductase activity of *Eleusine coracana* plants. *Current Botany* **18** (9), 33-36
- Jadhav R.K, Shital G. and Darshan S. (2018) Deproteinised forage juice (DPJ) stress influence on stomata and proline in gramineae crops. *Journal of Plant Physiology, (Photon) D-8947, In press.*
- Jadhav R.K. and Nimisha M. (2018) Protease inhibition by *Allium cepa*. L. forage deproteinised juice (DPJ) in *Trichoderma viride*. *Mycology and Plant Pathology*. **48** (3), 156-166.
- Jadhav R.K. and Mungikar A. M. (1998) Mitotic Inhibition and Chromosomal Abberations Induced by Deproteinised Leaf Juice Of *Medicago sativa*. L. in root tips of Onion (*Allium cepa*. L). *Int Journal Mendel*, **15** (1 - 2), 21.
- Jayanthi P., Lalitha P., and Sripathi S.K. (2011) Phytochemical investigation of the extracts of *Eichhornia crassipes* and its solvent fractionates. *Journal of Pharmacy Research*, **4** (5), 1405-1406
- Kothekar V. S. , Harsulkar A. M., Dadke R. D., Khandelwal A. R.(1995) Dominant traits in winged bean mutants. *The Journal of Cytology and Genetics*, **30**, 169
- Lara-Serrano J.S., Rutiaga-Quiñones O.M., López-Miranda J., Fileto-Pérez H.A., Pedraza-Bucio F.E., Rico-Cerda J.L., Rutiaga-Quiñones J.G. (2016) Physicochemical characterisation of Water hyacinth [*Eichhornia crassipes*. (Mart.) Solms], *Bioresources*, **11**(3), 7214-7223.
- Nikolova I, M. Georgieva, K. Kruppa, M. Molnár-Láng, L. Liu, V. Manova, L. Stoilov, (2015) Cytogenetic effects in barley root apical meristem after exposure of dry seeds to lithium ion beams. *Genetics and Plant Physiology*, **5** (1), 3–9.
- Nyananyo B.L, Gijo Ah, Ogamba, En. (2007) The physico - chemistry and distribution of water hyacinth (*Eichhornia crassipes*. L) on the river Nun in the niger nelta. *J. Appl. Sci. Environ. Manage*, **11** (3), 133- 137
- Oboh F. O, Ugheighele S and Odjadjare E. E. O. (2016). Fractionation and rational utilisation of *Vernonia amygdalina* leaves. *Journal of Basic and Applied Sciences*, **2** (1), 49 – 61.
- Satpute R. A. and Ghogre D. S. (2009) Mutagenic effects of gamma rays and sodium azide on frequency and spectrum of chlorophyll and macromutation in sunflower (*Helianthus annuus* L.). *Bioinfolet*, **6** (3), 193- 196
- Taha R.M. and Wafa S.N. (2012) Plant Regeneration and Cellular Behaviour Studies in *Celosia cristata* Grown *In Vivo* and *In Vitro*. *Scientific World Journal*, 359413
- Ying Tanga, Hai-liang Xina, Mei-li Guoa. (2016) Review on research of the Phytochemistry and pharmacological activities of *Celosia argentea*. *Brazilian Journal of pharmacognosy*, **26**, 787–796