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Chenopodium murale Leaf Aqueous Extract's Allelopathic Potential: Discovering via Mitotic Activity and Ultrastructural Analysis

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Abstract

Chenopodium murale's allelopathic potential was investigated by applying varying percentages of its leaf aqueous extract (LAE) to *Allium cepa* L. root tips for four days: 0.5%, 1%, 2%, and 4%. By looking at mitotic anomalies and the mitotic index (MI), the effect on mitotic activity was evaluated. Microscopic findings after *Chenopodium murale* LAE treatment showed a shift from normal mitoticity to abnormalities in Allium cepa root tip cells. This suggests that *Chenopodium murale* has allelochemicals that can interfere with *Allium cepa*'s regular mitotic cycle. The inhibitory effects of LAE that have been observed point to its potential as a natural weedicide that provides an environmentally friendly method of managing weeds in agriculture.

Key words: *Allium cepa* L., *Chenopodium murale* L., Leaf aqueous extract, Mitotic activity, Root tip cells

Despite being viewed as bothersome intruders, weeds have a complicated function in the natural world. These hardy plants, which are distinguished by their unwelcome presence in farmed regions, have unexpected advantages. In addition to being essential resources for pollinators and animals that consume seeds, weeds also improve soil health by fixing nitrogen and increasing water infiltration. They can also be used as markers of environmental imbalances. But because of their competition, agricultural productivity may face difficulties that need for cautious management techniques. Farmers can achieve a balance between conserving the fragile ecological web and increasing crop productivity by knowing the ecological roles of weeds and using focused control techniques.

The annual weed *Chenopodium murale* grows quickly, reaching heights of 60 cm and occasionally even 100 cm. At the base, the primary stem branches out in an upward manner. Its taproot is branching. The leaves are lobed, deltoid to rhombic-ovate, dark green, 1–10 cm long, and 1–6 cm wide. They have sharp teeth with uneven teeth on the margin (20 or more). With a long or short stalk, the base has a wedge form. Initially opposing, the lower leaves eventually become alternating. Older leaves are glossy and dark green above, whereas younger leaves have mealy undersides. Small, dense axillary and terminal panicles hold the inflorescence. According to reports, each plant can yield up to 24,000 seeds, and it spreads by seed production [1-3]. The seeds are small, spherical pits that are visible at high magnification on the surface. They are dull black, finely keeled, and have a diameter of 1.2–1.5 mm [4].

In Africa and India, *Chenopodium murale* is used as food; however, because of oxalates, its use is restricted [3]. Certain nutrients in food have the ability to bind to oxalates. In addition, it can be used as fodder, a potherb, and in sauces [5]. According to Komarov [6], Sweet [7], Tanaka [8], Kunkel [9], Facciola [10] and Moerman [11], the young shoots and leaves are consumed raw or cooked like spinach. The seeds can be ground into a powder and used with grains to create bread, among other baked goods. They are also edible [6,12,10,11]. Allergies can be brought on by the herb's pollen grains, though.

Many species of *Chenopodium* have a range of therapeutic benefits. There are several species of *Chenopodium* that are anthelmintic [13]. Vermifugal characteristics are present throughout the whole of *C. murale* [5]. Antihypertensive activity has been demonstrated by some flavonoids [14].

MATERIALS AND METHODS

Plants of *Chenopodium murale* were randomly gathered from natural stands spread over the Aligarh Muslim University campus in Aligarh, India. At an elevation of 178 meters, the Aligarh district stretches between 77°29' to 78°36' East longitude and 27°28' to 28°10' North latitude. After being cut off from the gathered plants, the leaves were dried in the shade. They were then oven-dried for 24 hours at 75°C to remove any residual moisture. After drying, the plant pieces were ground into a powder and kept in labelled polythene bags for further use.

A modified version of the technique used by Gulzar *et al*. [15] was utilized to prepare the extracts. For a whole day at room temperature, four grams of the dry powder were soaked in 100 millilitres of double-distilled water (w/v). The resulting extracts were filtered using Whatman No. 1 filter paper and three layers of muslin fabric in succession. After further diluting the resultant solution to produce 2%, 1%, and 0.5% solutions, it was used as a 4% stock solution. To solubilize the active hydrophilic molecules, cold treatment at 4°C was applied to all

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extracts (0.5, 1, 2, and 4%); this process lasted for 24 hours [16-17]. These extracts were used in bioassay investigations conducted in the lab. The leaf aqueous extracts shall be referred to as LAE from here on.

Squash technique

The mitotic cycle was studied in *Allium cepa* root tips as a model system because of the quantity of relatively large dividing cells, big chromosomes, and reduced chromosomal number (2n = 16) [18]. These chromosomes are perfect for observation since they stain nicely [19]. In February 2023, fresh onion bulbs of uniform size were chosen for cytogenetic analysis. Using the squash approach, the effect of LAE on mitosis was assessed in onion root tips [20]. To promote root formation, onion bulbs were cultivated in double distilled water (DDW) for four days. Freshly emerging roots were treated with 0.5%, 1%, 2%, and 4% LAE for a full day on the fifth day. DDW was used as the reference control. After the treatment was finished on the sixth day, root tips were removed and left for another 24 hours in a glacial acetic acid-ethyl alcohol (1:3, v/v) solution in order to eliminate any leftover debris. After three DDW washes, the fixed root tips were hydrolyzed for one minute at room temperature using 1 N HCl. After 30 minutes of acetocarmine staining, they were macerated in a single drop of 40% glacial acetic acid on a glass slide. A coverslip was placed on the glass slide, which was then sealed with clear nail paint. The experiment was repeated with five duplicates kept for each treatment. Comprehensive examination of several stages of mitosis was conducted using an optical source compound microscope (Olympus, model CH20i, New Delhi, India). The following formula was used to determine the mitotic index (MI):

$$MI = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells} \times 100$$

For every treatment, five slides had been generated and the average MI was determined.

Statistical analysis

Statistical analysis of the MI data was performed using SPSS/PC software version 16 (SPSS Inc., IL). Error bars in (Fig 1-2) represent the standard deviation of the measurements. Treatment means were statistically compared to the control using Duncan's Multiple Range Test (DMRT) at a p-value of less than 0.05 [21].

RESULTS AND DISCUSSION

Normal mitotic progression was disrupted when different amounts of LAE were applied to the root tips of Allium cepa plants. Although control samples showed normal mitotic phases, the frequency of aberrant mitotic stages rose as the quantity of LAE increased (Fig 1). A concentration-dependent decrease in the mitotic index (MI), a gauge of cell division activity, supported this pattern even further (Fig 2). At 4% LAE, the deviation from normal mitotic phases was the largest; at 0.5% LAE, the lowest anomalies were seen, and in the control group, total normality was evident (Table 1).

These results imply that LAE has the potential to be allelopathic, meaning it can disrupt cellular functions necessary for the growth and development of plants. The mitotic anomalies that have been found suggest that LAE could upset the delicate balance of cell division, which could result in cell death and growth retardation. To fully understand the exact processes by which LAE produces allelopathic effects and to investigate its potential as a natural weed control technique, more research is necessary.

Effect on mitotic index

Different quantities of LAE applied to the root tips of *Allium cepa* produced noticeable morphological changes as well as a major decrease in the mitotic index (MI). LAE-treated root tip cells showed abnormal nuclear orientations and irregular cell shapes in comparison to the control group (Plate 1). A concentration-dependent effect was seen in the frequency of mitotic abnormalities, which increased proportionately with LAE concentration (Fig 2). Furthermore, a significant decrease in MI was produced by LAE treatment at all dosages; the control group showed a MI of 16.81 ± 0.36, whereas the 0.5% and 4% LAE treatments showed a MI of 15.57 ± 0.34 and 8.25 ± 0.45, respectively (Fig 1). These results show that LAE has allelopathic potential, meaning it can cause disruptions to mitotic activity and cellular shape in root tip cells of *Allium cepa*. The anomalies that have been noticed imply that LAE might obstruct vital biological functions, which could result in cell death and growth retardation. In order to fully understand the mechanisms behind LAE's allelopathic effects and investigate its potential as a natural weed management technique, more research is necessary.

Plate 1 *Chenopodium murale* at flowering stage

Chromosomal abnormalities in onion root tip cells upon exposure to LAE

When comparing the normal mitotic cycle seen in control samples to that of *Allium cepa* root tip cells treated with different amounts of LAE, a variety of chromosomal aberrations were detected. LAE-treated cells showed a range of chromosomal abnormalities, but control cells showed regular chromosome organization and separation during metaphase, anaphase, and telophase. Chromosome sticking, multinucleated cell development, sickle-shaped cells, and membrane-damaged cells were among these anomalies (Plate 1). With an increase in LAE concentration, these abnormalities became more frequent; the maximum abnormality index was caused by 4% LAE (12.99 ± 0.47%), while the lowest abnormality index was caused by 0.5% LAE (3.33 ± 0.27%) (Fig 2).

Table 1 Effect on cell count of *Allium cepa* L. root tip cells at control and treated with various concentrations of LAE of *Chenopodium murale*

Treatments	Prophase cells count	Metaphase cells count	Anaphase cells count	Telophase cells count	Total cells counted
Control	42.37 ± 1.49	55.97 ± 1.18	45.25 ± 1.02	69.45 ± 1.08	1267.00 ± 50.54
0.5%	40.34 ± 1.35	46.48 ± 1.69	41.11 ± 1.02	64.41 ± 1.25	1235.67 ± 55.29
1.0%	38.42 ± 1.18	38.42 ± 1.09	44.33 ± 1.22	69.69 ± 1.47	1446.66 ± 38.18
2.0%	31.29 ± 1.18	30.60 ± 1.89	29.78 ± 1.02	53.52 ± 1.04	1244.00 ± 28.57
4.0%	12.17 ± 0.27	21.26 ± 1.89	29.27 ± 1.26	39.28 ± 1.18	1235.67 ± 24.78

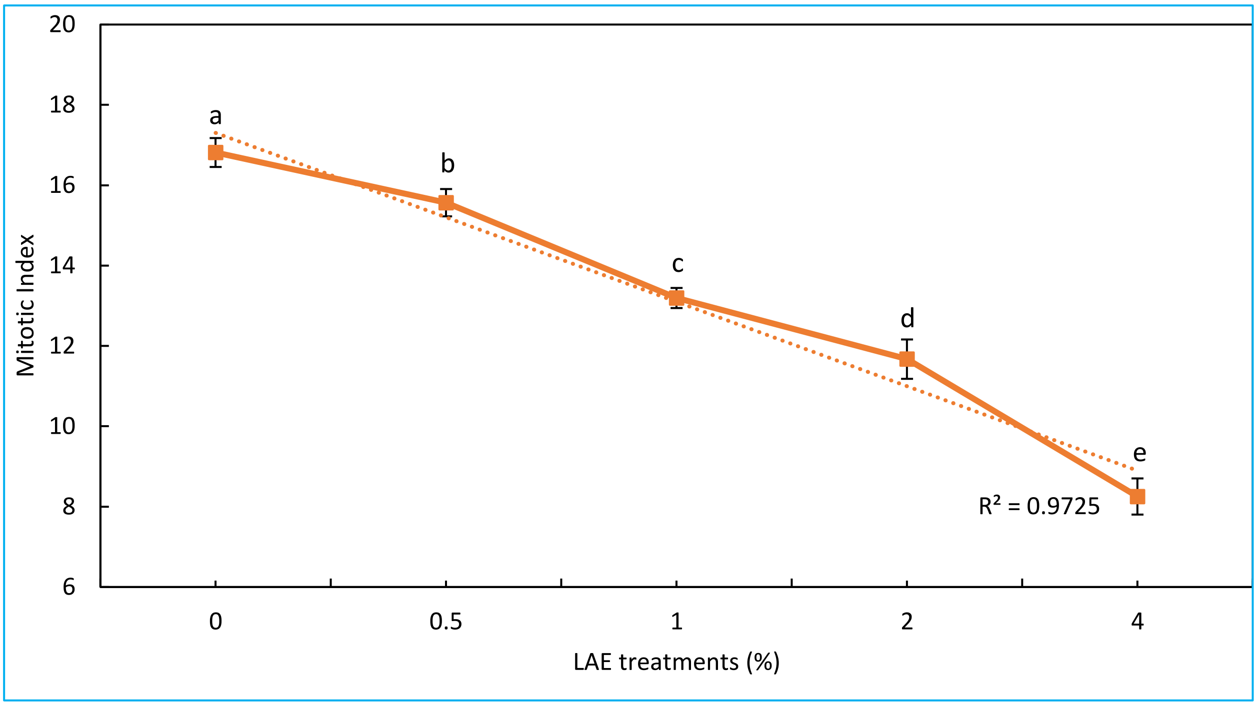

Fig 1 Mitotic index of *Allium cepa* L. root tip cells at control and treated with various concentrations of LAE of *C. murale*

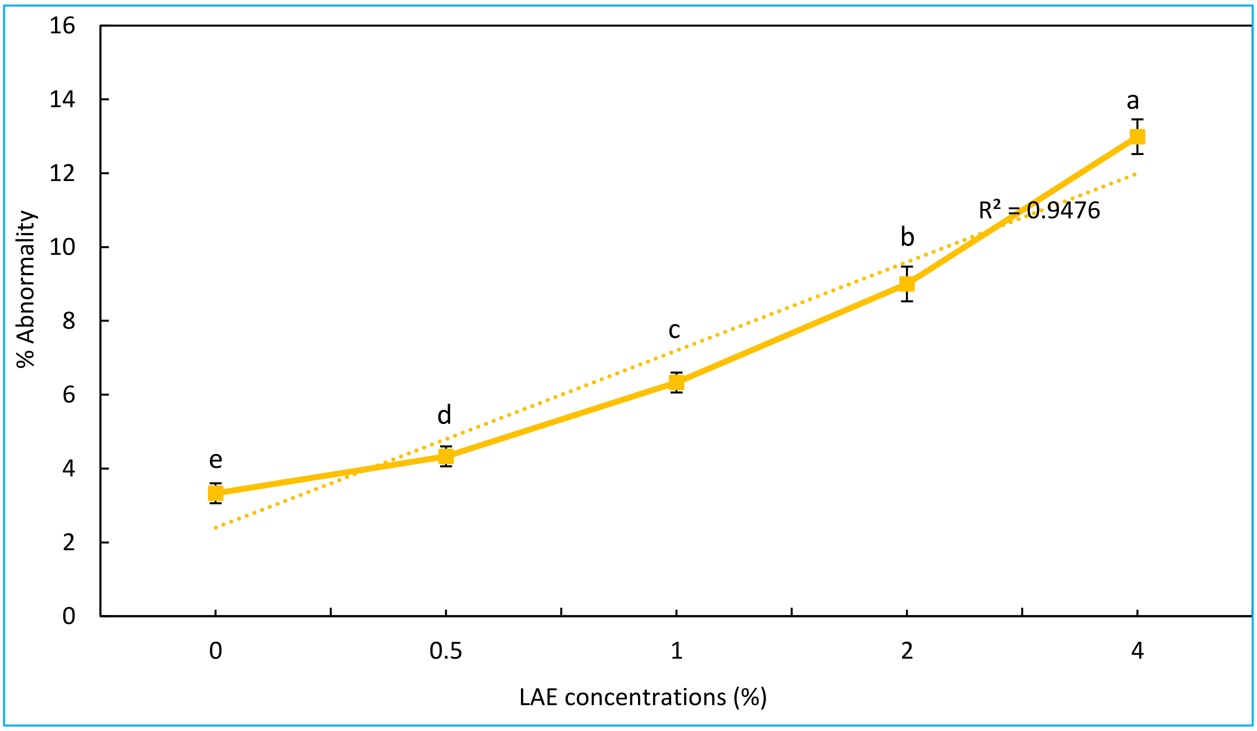

Fig 2 Abnormality index secured from *Allium cepa* L. root tip cells at control and treated with various concentrations of LAE of *C. murale*

Different alphabets in each curve (showing the effect of concentration) and different numerals among the curves at each concentration (showing the effect of different extract treatment) in each figure represent significant difference at $P<0.05$ applying DMRT (Duncan, 1955). R^2 represents value of correlation coefficient

The chromosomal abnormalities that have been found indicate that LAE may interfere with several phases of the mitotic cycle, possibly interfering with vital biological functions. Sticky chromosomes, chromosome bridges, and nuclear lesions were the most prevalent anomalies, suggesting possible impacts on DNA synthesis, chromosome segregation, and spindle formation. The existence of sickle-shaped and elongated cells implies that there is an effect on cell morphology. Together, these results show that LAE has allelopathic potential. It can impede mitotic process, which may result in growth retardation and cell death. To fully understand the precise mechanisms behind LAE's allelopathic effects and investigate its potential as a natural weed control technique, more research is necessary.

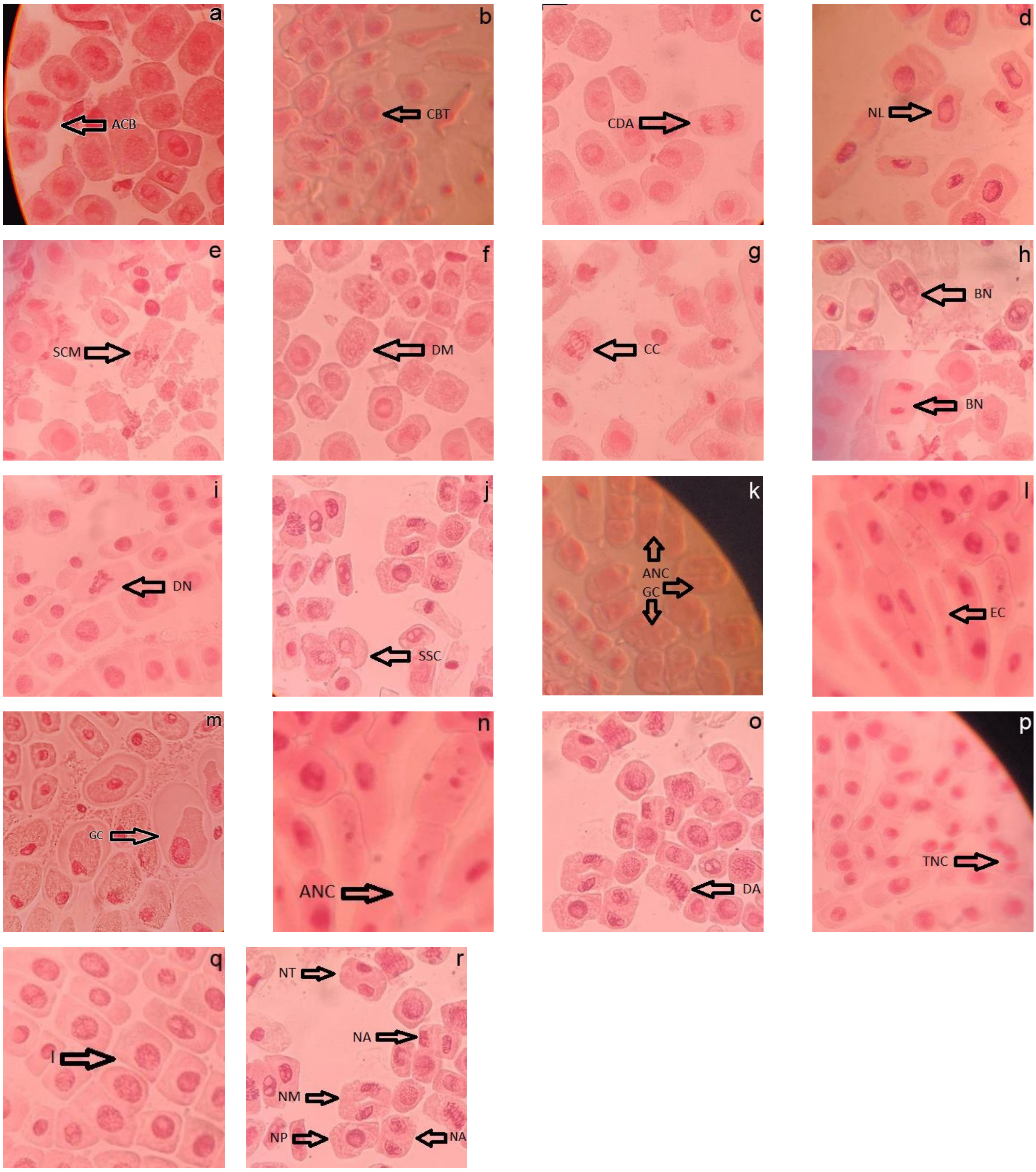

Plate 2 Alterations in genetics of *Allium cepa* L. showing different chromosomal aberrations incited when treated with different concentrations (0.5, 1, 2 and 4%) of leaf aqueous extracts (LAE) of *C. murale*. a-e: 1%; f-g: 2%; h-p: 4%; q-r: untreated control

ACB anaphase chromosome bridge, *CBT* chromosome bridge at telophase, *CDA* chromosome displacement at anaphase, *NL* nuclear lesion, *SCM* sticky chained metaphase in a polyploidy cell, *DM* disturbed metaphase, *CC* chromosome clump at metaphase, *BN* binucleated cell, *DN* deformed nucleus, *SSC* sickle shaped cells, *GH* ghost cells, *EC* elongated cells, *GC* giant cells, *ANC* anucleated cell, *DA* disturbed anaphase, *TNC* trinucleated cell, *I* interphase, *NP* normal prophase, *NM* normal metaphase, *NA* normal anaphase, *NT* normal telophase

Mitotic index (MI) significantly decreased after LAE treatment of *Allium cepa* root tips, suggesting that the compound can inhibit cell division (one-way ANOVA, $P<0.05$). Higher LAE concentrations (4%), where the inhibitory impact was more noticeable, indicate a concentration-dependent reaction [22-24]. These results are consistent with other research showing allelochemicals have inhibitory effects on the advancement of the cell cycle [22-23].

A range of chromosomal aberrations were also brought on by LAE treatment, indicating that it may interfere with different phases of the mitotic cycle [24]. Disrupted polarity, elongated cells, sickle-shaped cells, sticky chromosomes, chromosome displacement, chained chromosomes, anucleated cells, ghost cells, malformed nuclei, multinucleate cells and chromosome bridges were among these oddities [22-24]. These results are in line with earlier studies showing allelochemicals' aneugenic and clastogenic [23-24].

The chromosomal anomalies that have been found indicate that LAE may cause disruptions to vital cellular functions, which could lead to growth retardation and eventual cell death [24]. Although the exact processes causing LAE's allelopathic effects are yet unknown, the fact that LAE contains a variety of phenolic compounds raises the possibility that these chemicals may be involved in the disruption of cell division [15], [25]. To pinpoint the precise allelochemicals causing the effects that have been seen and to investigate LAE's potential as a natural weed control method, more research is necessary.

Allelochemicals included in *C. murale* leaf extract contribute to its phytotoxic properties. Phenolic chemicals are important in the allelopathic effects of *C. murale*, albeit they may not be the only factors contributing to allelopathic interactions in plants. Gulzar *et al.* [15] and Gulzar and Siddiqui [25] both provided evidence of the effect of phytochemicals containing phenolic acid on cytological aberrations in *Cassia sophera* L. and *Allium cepa* L. Similar findings were made by Banti and Hadjikakou [27] that *Allium cepa* root meristematic cells exhibit chromosomal abnormalities due to calix-4-arene derivatives.

Other allelochemicals found in *C. murale* leaf extract besides phenolic compounds have the ability to affect plant growth and development. According to [26] different phenolic derivatives have an impact on a range of physiological activities, including respiration, hormone balance, food uptake, membrane permeability, protein synthesis, photosynthesis, root elongation, and enzyme activity.

In order to determine the precise allelochemicals contained in the leaf extract of *C. murale* and to clarify their individual and combined impacts on plant growth and development, more research is necessary. The creation of environmentally acceptable defoliants for organic agricultural methods may be facilitated by this information.

CONCLUSION

Allium cepa root tips exhibit chromosomal abnormalities and disruptions in cell division due to *Chenopodium murale* extract, indicating the possibility of allelopathy. The allelopathic effects of *C. murale* are ascribed to the existence of several phytochemicals, such as derivatives of calix-4-arene and phenolic compounds. It has been demonstrated that these substances cause cell division problems and chromosomal abnormalities in a variety of plant species. This suggests the existence of naturally occurring allelochemicals that could be used to manage weeds in an environmentally responsible manner. Using *C. murale* extract as a natural weed killer has a few potential benefits over traditional synthetic herbicides. Natural allelochemicals are more environmentally friendly since they are typically less hazardous to creatures other than their intended targets and less enduring in the environment. Furthermore, using natural weedicides may help maintain the health of ecosystems and soil biodiversity. In order to create targeted and efficient natural herbicides from *C. murale* extract for sustainable agricultural practices, more research is necessary to discover and describe these allelochemicals.

LITERATURE CITED

1. Parker KF. 1972. *An Illustrated Guide to Arizona Weeds.* The University of Arizona Press, Tucson, AZ. Available online at http:// www.uapress.arizona.edu/online.bks/weeds.
2. Herron J. 1953. *Study of Seed Production, Identification and Seed Germination of Chenopodium spp.* Cornell University Agricultural Experiment Station, Memoir 320, Cornell University, Ithaca, USA.
3. Holm, LeRoy, Doll J, Holm E, Pancho JV, Herberger JP. 4997. *World weeds: Natural histories and distribution*. John Wiley & Sons.
4. Wagner WL, Herbst DR, Sohmer SH. 1999. *Manual of the Flowering Plants of Hawaii*. Revised edition. University of Hawai'i Press, Honolulu, USA.
5. Phondke GP. 1992. *The Wealth of India - Raw Materials*. Vol. III. NISCAIR, IARI, New Delhi. pp 471-471.
6. Komarov VL. 1968. *Flora of the USSR.* Israel Program for Scientific Translation.
7. Sweet M. 1962. *Common Edible and Useful Plants of the West*. Nature Graph Co, USA.
8. Tanaka T. 1976. *Tanaka's Cyclopaedia of Edilble Plants of the World.* Keigaku Publications (in Japanese with English summary).
9. Kunkel G. 1984. *Plants for Human Consumption.* Koeltz Scientific Books.
10. Facciola S. 1990. *Cornucopia - A Source Book of Edible Plants*. Kampong Publications.
11. Moerman DE. 1998. *Native American Ethnobotany*. Portland, Oregon: Timber Press.
12. Yanovsky E. 1990. Food Plants of the North - American Indians. Publication No. 237. U.S. Department of Agriculture.
13. Lozoya J, Lozoya M. 1982. *Flora Medicinal de Mexico. Primera Part: Plantas in Digenas.* Instituto Mexicano del Seguro Social, Mexico. pp 31.
14. Ahmad AG, Elmazar MMA. 1997. Isolation of hypotensive flavonoids from *Chenopodium species* growing in Egypt. *Phytotherapy Research* 11: 564-567.
15. Gulzar A, Siddiqui MB, Ansari S. 2016. Phenolic acid allelochemicals induced morphological, ultrastructural, and cytological modification on *Cassia sophera* L. and *Allium cepa* L. *Protoplasma* 253(5): 1211-1221.
16. Borghetti F, Silva L, Pinheiro JD, Varella BB, Ferreira AG. 2005. Aqueous leaf extract properties of Cerrado species in Central Brazil. *In*: (Eds) Harper JDI, An M, Wu H, Kent JH. Proceedings and selected papers: Fourth World Congress on Allelopathy. The George Stutt University, Wagga. pp 388-390.

17. Pina GDO, Borghetti F, Silveira CES, Pereira LAR. 2009. Effects of *Eugenia dysenterica* leaf extracts on the growth of sesame and radish. *Allelopathy Journal* 23: 313-322.
18. Havey MJ. 2002. Genome organization in *Allium*. *In*: (Eds) Rabinowitch. H. D., Currah, L. *Allium* Crop Science. Recent Advances. CABI Publishing, United Kingdom. pp 59-79.
19. Fiskesjo G. 1997. *Allium* test for screening chemicals: evaluation of cytologic parameters. *In*: (Eds) Wang, W., Gorsuch, J. W., Hughes, J. S. Plants for Environmental Studies. CRC Publishers, Boca Raton. pp 308-333.
20. Mushtaq W, Ain Q, Siddiqui MB, Hakeem KUR. 2019. Cytotoxic allelochemicals induce ultrastructural modifications in *Cassia tora* L. and mitotic changes in *Allium cepa* L.: A weed versus weed allelopathy approach. *Protoplasma*. https://doi.org/10.1007/s00709-018-01343-1
21. Duncan DB. 1955. Multiple range and multiple F-tests. *Biometrics* 11: 1-42.
22. Soltys D, Rudzinska-Langwald A, Gniazdowska A, Wisniewska A, Bogatek R. 2012. Inhibition of tomato (*Solanum lycopersicum* L.) root growth by cyanamide is due to altered cell division, phytohormone balance and expansin gene expression. *Planta* 236(5): 1629-1638.
23. Devi S, Thoppil JE. 2016. Cytotoxic studies and phytochemical analysis of *Orthosiphon thymiflorus* (Roth) sleesen. *International Journal of Pharmacy and Pharmaceutical Sciences* 8(2): 249-255.
24. Silva ALN, de Almeida AA, de Araújo JL, dos Santos MR, da Silva MG. 2022. Allelopathic effects of leaf and root extracts of *Chenopodium quinoa* on *Allium cepa* L. root meristems. *Ciência Rural* 52(2).
25. Gulzar A, Siddiqui MB. 2014. Evaluation of allelopathic effect of *Eclipta alba* (L.) Hassk on biochemical activity of *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L. *African Journal of Environmental Science and Technology* 8(1): 1-5.
26. Liu Q, Lu D, Jin H, Yan Z, Li X, Yang X, Qin B. 2014. Allelochemicals in the rhizosphere soil of *Euphorbia himalayensis*. *Journal of Agricultural and Food Chemistry* 62(34): 8555-8561.
27. Banti P, Hadjikakou SK. 2019. Genotoxic effects of calixarene derivatives on *Allium cepa* L. root meristematic cells and normochromatic erythrocytes (NCEs) of Balb/c mice. *Food and Chemical Toxicology* 128: 145-153.