

Integrated Study on Pesticide Residue Detection in Okra By LC–MS/MS and Biological Pest Management Through Plant Derived Extracts

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ABSTRACT

Vegetable farming holds a significant position in Indian agriculture due to the nutritional, medicinal, and commercial value of the produce. Okra is consumed as a green vegetable and is highly nutritious, serving as an excellent source of vitamins C and K, and protein, while being low in calories. The present study utilized LC-MS/MS to analyze pesticide residues in okra. The analysis revealed that the okra samples were contaminated with pesticide residues exceeding the Maximum Residue Limits (MRLs), rendering them unsafe for consumption. This contamination underscores the urgent need to implement biological management strategies as sustainable and safer alternatives to traditional pesticide use. This study also focused on the role of biological management strategies as sustainable alternatives. This study evaluated the efficacy of leaf extracts of four aromatic plants i.e. Lantana camara, Mentha piperita, Cymbopogon citratus and Cymbopogon martinii against the pathogens in okra. In terms of growth parameters, Lantana camara and Mentha piperita treated plants also showed increased plant height, higher leaf number and total fresh biomass. This suggests their dual role in pathogen suppression and growth promotion. Notably, these treatments also significantly improved yield. It gives eco-friendly and natural, alternative to synthetic pesticides, with added advantage of minimal toxicity to the non-target organisms and biodegradability, this makes it a valuable asset in biocontrol and sustainable agriculture systems. The present work on the analysis of pesticides residue in fruit vegetables and evaluation of biological control by using plant extracts has provided valuable insights into both sustainable agriculture and food security.

Keywords: Fruit vegetable, LC–MS/MS, pesticide residues, biological control, Okra.

INTRODUCTION

India is a predominantly agricultural country with fertile plains. A large variety of vegetables and fruits are cultivated here. Farmers work day and night to produce a variety of agricultural crops, vegetables and fruits and play an important role in earning foreign exchange for the country. The production of high-quality fruit vegetables like tomato, potato, bitter melon, ridged guard, brinjal, okra, chilli, cucumber, cauliflower, etc. is very beneficial to the country's economy. There are many types of mites, insects and plant pathogens that attack vegetable and fruit crops in different parts of India and cause great damage. The insect pests are also played very important role in spreading these diseases from diseased plants to the healthy plants. These insects prefer and feed on the young parts of the plant and some insects bore holes

in the fruits and lay eggs in them, causing damage to the fruit vegetable (Ankireddy et al., 2023; Ansari et al., 2019; Parthasarathy et al., 2024). Some of the most common and economically important sucking insects include aphids, whiteflies, leafhoppers, psyllids, thrips, mealybugs, cotton bugs, shield bugs and spider mites, etc. (Ghosal, 2022; Saha et al., 2020).

Okra is a worldwide cultivated vegetable crop for its nutritional and economic importance. But their productivity is significantly reduced by number of diseases caused by bacterial, fungal and viral pathogens. Farmers use pesticides to control weeds that compete with crops, crop diseases, and try to increase their productivity through their use. It includes herbicides, fungicides and insecticides are routinely used during the cultivation and post-harvest

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processing of fruits and vegetables. However, the widespread and often improper use of pesticides has led to contamination of vegetables and fruits with pesticide residues, posing serious human health and food safety problems. The spread of agrochemical pollutants to environmental and agricultural lands has led to devastating ecosystem pollution (i.e., dust, air, soil, sediment, and water) and loss of human food supplies worldwide (Kumar et al., 2018). The World Health Organization reports about 3 million annual cases of pesticide poisoning and up to 220,000 deaths, mainly in developing countries (Sola et al., 2014). According to WHO, 2020, "Pesticide residues in food are of international concern due to their potential adverse health effects, particularly on vulnerable populations such as children and pregnant women." Fruits and vegetables, which are frequently and often eaten raw, serve as an important source of pesticide exposure for the general population. Studies around the world have consistently reported pesticide residues in fresh vegetable and fruit products, most of which are above the MRLs (maximum residue limits) set by different regulatory bodies such as the WHO/FAO Codex Alimentarius Commission. Research has found that chronic exposure to certain chemicals and pesticides has been associated with an increased incidence of prostate, breast, bladder, lung, colon leukemia, and multiple myeloma cancers (Ahmed et al., 2017). In addition to human health, the use of pesticides also poses significant risks to the environment. Agricultural and commercial growth have released large amounts of pesticides and other chemicals into the environment (Varjani et al., 2018). The rapid expansion of the world population also underscores the need to maximize food supplies. Furthermore, the widespread use of these pesticides has led to a range of negative impacts, from environmental pollution to ecosystem destruction (Barbieri et al., 2019; Kock Schulmeyer et al., 2019). Pesticide residues can leach into soil and water supplies and disrupt microbial diversity, and can harm non-target organisms such as aquatic animals, pollinators and birds. UNEP (2021) warned that "pesticides cause damage to biodiversity, air, water and soil pollution, which reduces ecological resilience and sustainability." There is an urgent need for safer alternatives and more responsible pest management practices.

In recent years, global concerns for environmental protection have led researchers to investigate the use of natural plants as a source of crop protection (Wijewardane and Guleria 2009).

MATERIAL AND METHODS

Study Area:

Nanded is a district in Maharashtra, India, strategically located on the border shared with the states of Karnataka and Telangana. Geographically, the district is bordered by Karnataka to the south-west and Telangana to the south. The study area spans from 18°15' to 19°55' North latitude and 77° to 78°25' East longitude. The total area of Nanded District is 10,444 square kilometers, which constitutes 3.41% of the total area of Maharashtra state. The region boasts numerous large fresh produce markets, and vegetables are readily available through street vendors, alongside several farms dedicated to cultivating fruit vegetables consumed within the district. Furthermore, the district has an urban population of 30%, which accounts for a substantial consumption of the fruit vegetables produced locally (Biswas, B., & Kaplay, R. D. 2012).

Collection of Okra Samples for Screening of Multi Pesticides Residue Analysis:

The okra samples were collected from selected locations and monitored twice a year for three consecutive years from January 2023 to July 2025. A total of 20 okra samples were collected from small farms, community markets, households and street vendors in Nanded district between January 2023 and July 2025. The okra samples (1kg) were collected, packed and labelled from different locations. The samples brought in to the laboratory and kept in cold until they used. The samples were collected and mixed and then ground.

Pesticide Residue Analysis by QuEChERS Method:

The fruit vegetable sampling and cleaning were performed using the QuEChERS method (Quick, Easy, Cheap, effective, rugged and safe). The QuEChERS method is based on the work performed and published by Anastassiades et al., 2003. The QuEChERS method was developed using an



extraction method for pesticides in fruit vegetables, which included a clean-up method that removed sugars, lipids, organic acids, sterols, proteins, pigments and excess water. The method offers a convenient alternative to traditional liquid-liquid and solid phase extractions. The process involves two simple steps. First, homogenized samples are extracted and fractionated using organic solvents and salt solutions. Then, the supernatant is further extracted and cleaned using a dispersive solid phase extraction (dSPE) technique. The collected fresh vegetable samples (1 kg) were chopped and ground in a blender. Fifteen grams of each sample (3 replicates) were used for multi-residue analysis by the QuEChERS method. (15 g) sample was mixed in 30 ml acetonitrile and 3 g NaCl was added, centrifuged at 2500 rpm and then sodium sulfate was added to remove the water content and vortexed at 50 rpm for ten minutes. The extracted sample was centrifuged at 10,000 rpm for ten minutes. Nine ml of supernatant extract was cleaned with a mixture of 0.4 g PSA, 1.2 g anhydrous MgSO₄. This extract was again shaken with a cyclo mixer at 50 rpm for 5 minutes and centrifuged at 10,000 rpm for ten minutes. The supernatant (2 ml) was taken and evaporated with a turbo vap and finally made up to 1 ml with hexane. One microliter was used for analysis on GC MS/MS.

Biological Management of Pest

These experiments were carried out in Nanded District, Maharashtra, during 2024 to 2025, to evaluate the potential of different aromatic plant against Okra (*Abelmoschus esculentus* (L.) Moench) for controlling fruit vegetable pests and their diseases.

Collection of Plant Materials:

During 2024 to 2025, fresh leaves were collected from disease-free plants of potential biopesticides such as *Lantana camara*, *Mentha piperita*, *Cymbopogon citratus* and *Cymbopogon martini*. The collected plant samples were later identified taxonomically by the Department of Botany, Yeshwant College, Nanded.

Field Design

The selected fruit vegetable crop seedlings i.e. Okra (*Abelmoschus esculentus* (L.) Moench), was planted by randomized block design with three replications.

The size of plot was 3 x 2 square meter, with 20 plants / plot. Plant to plant and row to row distance was maintained 50 cm and 100 cm respectively. Standard agronomic and cultural practices were applied uniformly to all experimental plots.

Treatments and its Application

The extracts of all selected aromatic plants and standard (Insecticide) were sprayed twice per week by Knap Sack sprayer at 10 days interval.

Preparation of Plant Extract

Plant leaves of Lantana (*Lantana camara* L.), lemon-grass (*Cymbopogon citratus* (DC) Stapf), rosha grass (*Cymbopogon martinii* (Roxb) Wats.), peppermint (*Mentha piperita* L.), were collected from rural region of District Nanded. Before grinding or cutting, the leaves were dried up in lab for 8 to 10 days. Ten percent (10%) stock solution of each plant extract was prepared by mixing 100 gm of leaf powder in one litre of tap water and kept for three days. The mixture was thoroughly shaken, left for 24h and filtered through muslin cloth to remove the impurities and preserved the filtrate in refrigerator until use. 10% concentration for field application was prepared from the stock solution.

Spray and Monitoring

The botanical solution was sprayed on selected fruit plants experimental field twice a week with the help of a sprayer. The pest was monitored every day and damages were counted every 3 days in a week. The numbers of infested leaves were also recorded.

RESULT AND DISCUSSION:

Assessment of Pesticide Residues in Okra Fruit Vegetable.

Six different pesticide residues were found while analyzing the Okra fruit vegetable samples by GC-MS/ LC-MS presented in Table 1, viz. Cyazofamid, Difenconazole, Fluopyrum, Mandipropamid, Metrafenone, and Spirotetramat and Spirotetramat-enol. Cyazofamid (0.917 mg/ml), Difenconazole (0.131 mg/ml), and Mandipropamid (0.121mg/ml) are found to exceed their MRLs (Maximum Residue



Limits) 0.01 mg/ml, 0.05 mg/ml and 0.01 mg/ml respectively, showing serious insubordination with food safety standards.

Other three like Fluopyrum, Metrafenone, and Spirotetramat and Spirotetramat- enol were present at 0.253 mg/ml, 7.455 mg/ml, 2.45 mg/ml respectively and showed more values than their LOQ. These pesticides have no established MRLs value available,

hence comparison is not possible. All the detected values were above the limit of quantification (LOQ) of 0.01 mg/ml, which proves their reliable quantification. Due to these significant violations, the sample is considered unsafe for consumption. The graphical presentation, chromatogram and compound graphics of pesticide analysis of Okra fruit vegetable are shown in Figures 1, 2 and 3, respectively.

Table 1. Determination of pesticide residue levels in Okra (*Abelmoschus esculentus*) fruit vegetable by GC-MS/ LC-MS.

Sr. No.	Pesticides	Pesticide Values Observed (mg/ml)	MRL Values (mg/ml)	LOQ (mg/ml)	Pesticides above MRL (mg/ml)
1.	Cyazofamid	0.917	0.01	0.01	Yes
2.	Difenoconazole	0.131	0.05	0.01	Yes
3.	Fluopyrum	0.253	NA	0.01	NA
4.	Mandipropamid	0.121	0.01	0.01	Yes
5.	Metrafenone	7.455	NA	0.01	NA
6.	Spirotetramat and Spirotetramat- enol	2.45	NA	0.01	NA

Note: BLQ: Below the Level of Quantification, LOQ: Limit of Quantification, NA: MRLs Not Available

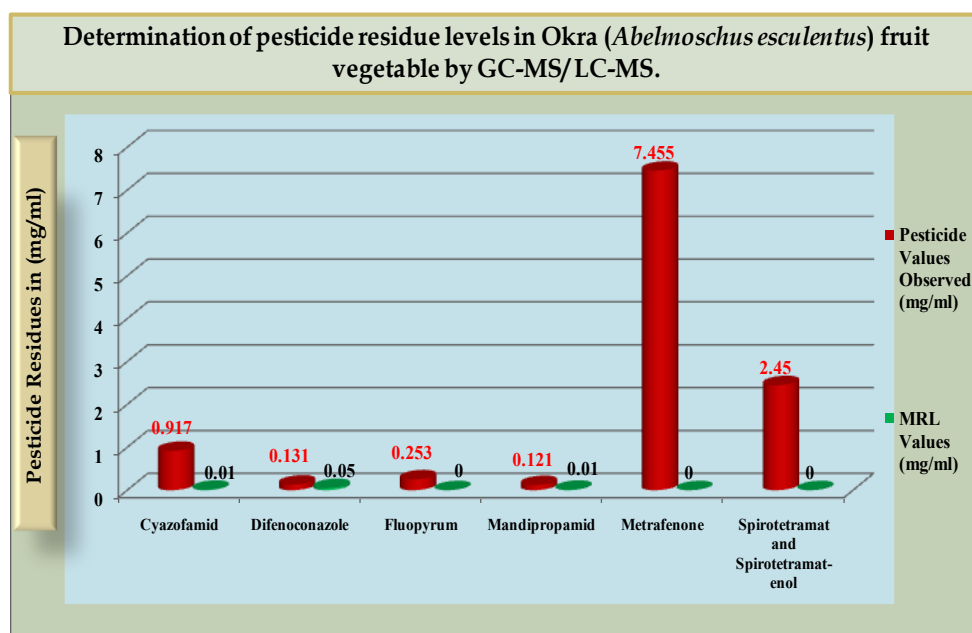


Figure 1. Determination of pesticide residue levels in Okra (*Abelmoschus esculentus*) fruit vegetable by GC-MS/ LC-MS.

Quantitation Results				
Sr.no.	Compound	RT	Response	Final Concentration
1.	Spirotetramat Enol	11.524	105547	27.2659 ng/ml
2.	Mandipropamid	13.356	308254	121.4811 ng/ml
3.	Fluopyram	13.937	491005	253.2708 ng/ml
4.	Spirotetramate	14.031	288912	212.3006 ng/ml
5.	Cyazofamid	14.157	58256	917.1677 ng/ml
6.	Metrafenone	15.257	21733844	7454.8229 ng/ml



7.	Difenconazole	15.319	370267	131.2406 ng/ml
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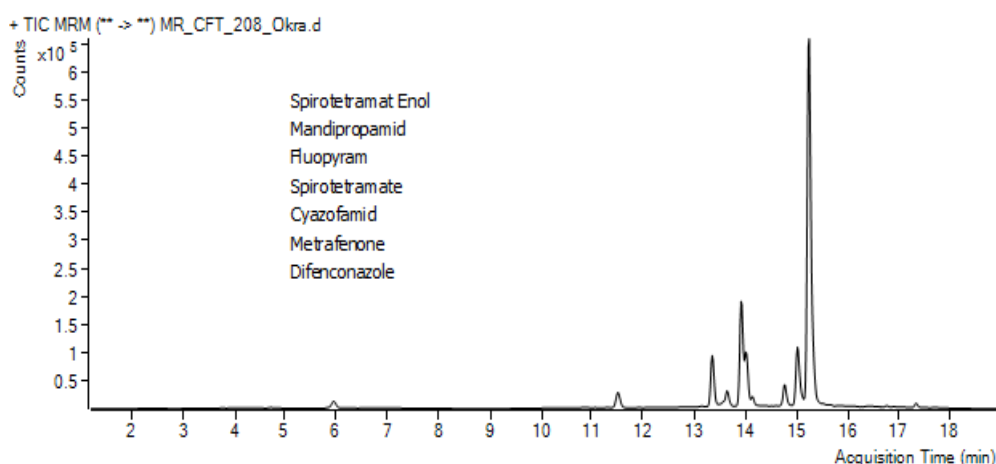


Figure 2: GC-MS/LC-MS chromatogram of Pesticide residues analyzed from Okra (*Abelmoschus esculentus*) fruit sample.

Biological Management

Effect of Selected Plant Extract on Crop Yield and Pest Control in *Abelmoschus esculentus* (L.) Moench.

Table 2. Screening of selected plant leaf extracts on *Abelmoschus esculentus* (L.) Moench against pest attack.

Sr. No	Treatments	Concentrations	No. of Infestation Leaves
1.	<i>Lantana camara</i>	10%	3.00±0.57
2.	<i>Cymbopogon citratus</i>	10%	8.66±1.76
3.	<i>Cymbopogon martinii</i>	10%	15.00±1.52
4.	<i>Mentha piperita</i>	10%	4.66±1.20
5.	Control (Untreated)	10%	27.33±1.45

In present study, the effects of four selected plant extracts on *Abelmoschus esculentus* were tested in an experimental field. Most of all the plant extracts showed good pesticide potential against fruit vegetable pest of selected fruit vegetable crops for study, the results are presented in Table 2. Out of them *Lantana camara* leaves extract (3.00±0.57) showed best pesticide potential against the pest attack on *Abelmoschus esculentus* leaves as compare to control

(Untreated) (27.33±1.45). *Mentha piperita* leaf extract was also found very effective (4.66±1.20).

The efficacy of *Cymbopogon citratus* (8.66±1.76) and *Cymbopogon martinii* (15.00±1.52) leaf extracts were exhibited nearly same effect in *Abelmoschus esculentus* plot against the pest attack. Graphical presentation of pesticide potential of selected plant leaves against fruit pest of *Abelmoschus esculentus* is depicted in Figure 4.

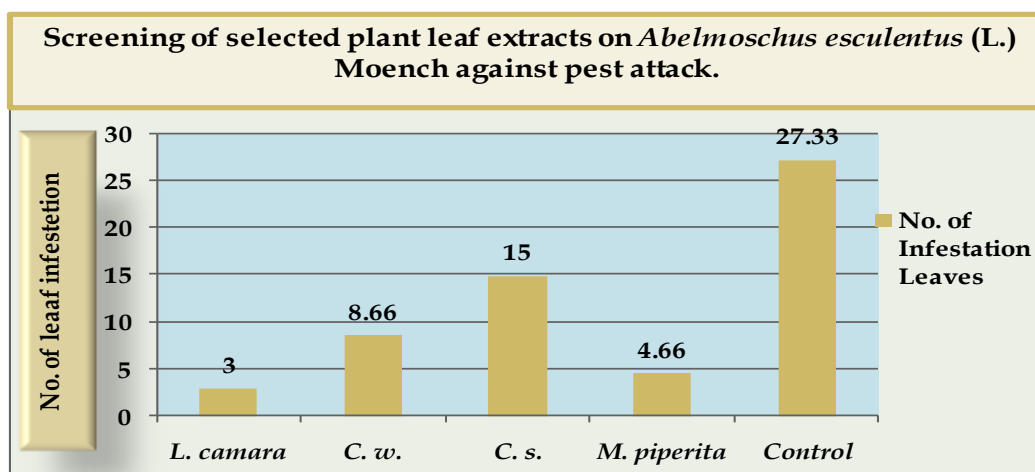


Figure 4. Screening of selected plant leaf extracts on *Abelmoschus esculentus* (L.) Moench against pest attack.

Table 3. Biopesticidal potential of selected plant leaf extracts against foliage damage in *Abelmoschus esculentus* (L.) Moench field.

Sr. No	Treatments	No. of Perforation in Leaves/Plant	% Reduction in Damage/Plant	Effectiveness Rank
1.	<i>Lantana camara</i>	5.66±1.20	63.07	1
2.	<i>Cymbopogon citratus</i>	8.00±2.00	47.81	3
3.	<i>Cymbopogon martinii</i>	13.33±2.72	13.04	4
4.	<i>Mentha piperita</i>	7.33±0.88	52.18	2
5.	Control (Untreated)	15.33±0.88	-	-

In present study, the comparative bio-pesticide potential of leaves of four selected plants *Lantana camara*, *Cymbopogon citratus*, *Cymbopogon martinii*, *Mentha piperita* L., against leaf perforation observed in *Abelmoschus esculentus* plants under field conditions. The results are presented in (Table 3). The result showed the mean number of perforations per leaf, percentage reduction in leaf

damage and effectiveness rank compared to the untreated control. The result indicates varying levels of bio-pesticidal potential, with all selected plant leaf extracts. The extract was applied as a foliar spray at a standard concentration of 10% after every 10 days. Each treatment was repeated three times under a randomized block design. The per-cent of reduction was calculated by formula: % Reduction in Damage = $[(\text{Control} - \text{Treatment}) / \text{Control}] \times 100$.

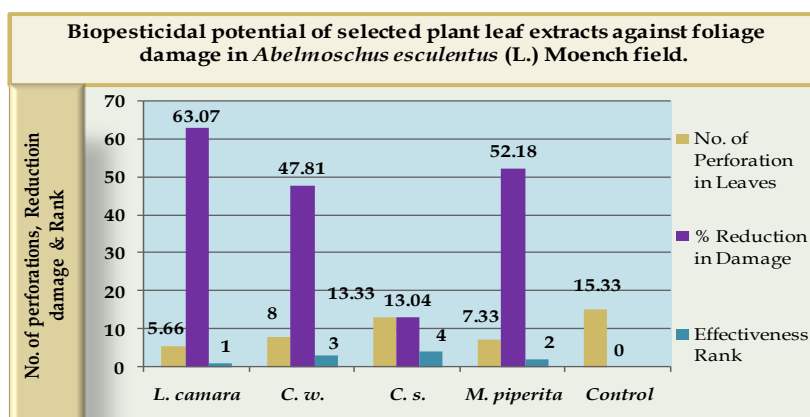


Figure 5. Biopesticidal potential of selected plant leaf extracts against foliage damage in *Abelmoschus esculentus* (L.) Moench field.

Most of all the plant extracts showed bio-pesticide potential against fruit pest of selected fruit crops for study. Out of them *Lantana camara* leaves extract (5.66 ± 1.20) showed best performance against the pest attack on *Abelmoschus esculentus* leaves as compare to untreated control (15.33 ± 0.88). *Mentha piperita* (10%) leaf extract was also found very effective

against pest and number of perforation was decreased (7.33 ± 0.88). *Cymbopogon citratus* then showed fewer perforations (8.00 ± 2.00) and among all the leaf extracts used, *Cymbopogon martinii* showed the lowest efficacy (13.33 ± 2.72). Graphical presentation of pesticide potential of selected plant leaves against fruit pest of *Abelmoschus esculentus* is depicted in Figure 5.

Table 4. Effect of selected plant extracts on height, leaf and biomass of *Abelmoschus esculentus* (L.) Moench field.

Sr. No.	Treatments	Length of Plants (cm)	Number of Leaves/Plant	Total Fresh Biomass (gm/plant)
1.	<i>Lantana camara</i>	125.33 ± 3.17	20.33 ± 0.88	304 ± 4.04
2.	<i>Cymbopogon citratus</i>	112.33 ± 1.45	15.00 ± 1.15	251 ± 3.15
3.	<i>Cymbopogon martinii</i>	114.66 ± 1.45	13.33 ± 0.88	256 ± 5.33
4.	<i>Mentha piperita</i>	118.66 ± 2.33	14.66 ± 0.88	281 ± 2.46
5.	Control (Untreated)	103.78 ± 2.18	12.33 ± 1.45	239 ± 9.60

The result presented in the Table 4 shows the comparative effect of leaves of four selected plants *Lantana camara*, *Cymbopogon citratus*, *Cymbopogon martinii*, *Mentha piperita* L., and the untreated control on length of plants, number of leaves and total fresh biomass of *Abelmoschus esculentus*. The extract was applied as a foliar spray at a standard concentration of 10% every 10 days after flowering. Results were collected at the time of harvest and averaged across three replicates. The results showed that *Lantana camara* leaf extract showed more length of plants, number of leaves and total fresh biomass per plant i.e. 125.33 ± 3.17 cm/plant, 20.33 ± 0.88 and 304 ± 4.04 gm/plant respectively. *Mentha piperita* leaf extract was also found very effective and showed more length

of plants, number of leaves and total fresh biomass per plant i.e. 118.66 ± 2.33 cm/plant, 14.66 ± 0.88 and 281 ± 2.46 gm/plant respectively.

Followed by *Cymbopogon martinii* and *Cymbopogon citratus* showed least effect as compared to other plant extracts. *Lantana camara* extracts significantly improved the productivity of *Abelmoschus esculentus*, yielding the highest length of plants, number of leaves and total fresh biomass, probably due to their effective pest control and growth-promoting properties. Graphical presentation of pesticide potential of selected plant leaves against fruit pest of *Abelmoschus esculentus* is depicted in Figure 6.

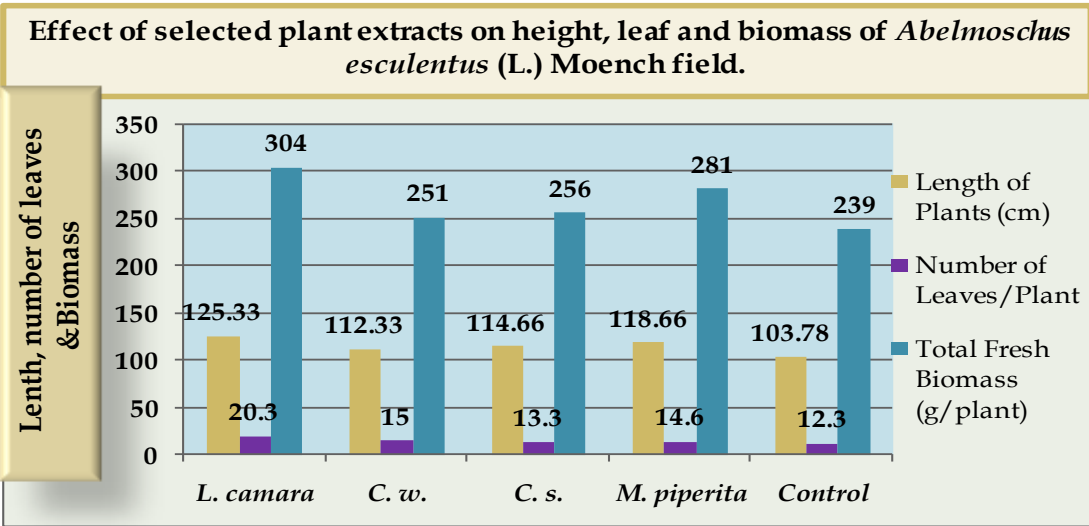


Figure 6. Effect of selected plant extracts on height, leaf and biomass of *Abelmoschus esculentus* (L.) Moench field.

Table 5. Impact of selected plant extracts on fruit number, weight, and total yield in *Abelmoschus esculentus* (L.) Moench field.

Sr. No	Treatments	Number of Fruits/ Plant	Fruit Weight (gm/plant)	Total Yield (kg/ Plot)
1.	<i>Lantana camara</i>	24.66±3.17	143.33±6.00	1.52±3.12
2.	<i>Cymbopogon citratus</i>	20.33±1.33	125.33±2.60	1.29±2.66
3.	<i>Cymbopogon martinii</i>	14.66±1.54	108.33±1.20	1.14±4.23
4.	<i>Mentha piperita</i>	20.66±1.76	127.66±1.76	1.39±2.15
5.	Control (Untreated)	11.00±1.15	106.66±4.40	1.20±1.45

The result presented in the Table 5 shows the comparative effect of leaves of four selected plants *Lantana camara*, *Cymbopogon citratus*, *Cymbopogon martinii*, *Mentha piperita* L., and the untreated control on fruit number, fruit weight and total yield of

Abelmoschus esculentus. The extract was applied as a foliar spray at a standard concentration of 10 percent every 10 days after flowering. The results were collected at the time of harvest and averaged across three replicates.

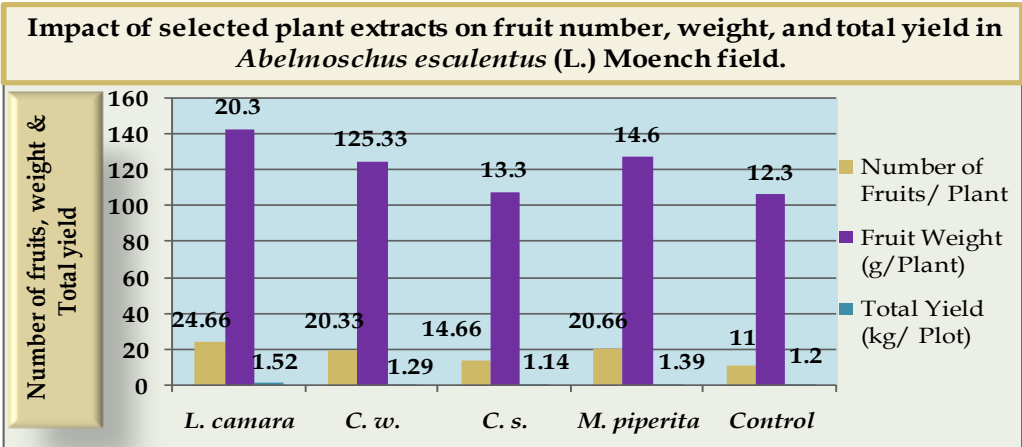


Figure 7. Impact of selected plant extracts on fruit number, weight, and total yield in *Abelmoschus esculentus* (L.) Moench field



The results showed that *Lantana camara* showed highest number of fruits per plant i.e. 24.66 ± 3.17 , fruit weight per plant was 143.33 ± 6.00 gm/plant and total yield was 1.52 ± 3.12 kg/plot. *Mentha piperita* leaf extract was also found very effective and showed number of fruits per plant i.e. 20.66 ± 1.76 , fruit weight per plant i.e. 127.66 ± 1.76 gm/plant and total yield was 1.39 ± 2.15 kg/plot. Followed by *Cymbopogon citratus* showed number of fruits per plant i.e. 20.33 ± 1.33 , fruit weight per plant i.e. 125.33 ± 2.60 gm/plant and total yield was 1.29 ± 2.66 kg/plot. While *Cymbopogon martinii* showed least effect as compared to other plant extracts. Graphical presentation of pesticide potential of selected plant leaves against fruit pest of *Abelmoschus esculentus* is depicted in Figure 7.

Lantana camara extracts significantly improved the productivity of *Abelmoschus esculentus*, resulting in the highest number of fruits and yields per plot, probably due to their effective pest control and growth promoting properties.

CONCLUSION

The present study highlighted the importance of pesticide residue detection in okra. It highlighted how pesticide levels in some products can exceed permissible limits, making them unsafe for consumption. Okra showed the highest contamination, with Cyazofamid, Mandipropamid and Difenconazole exceeding the MRL, in addition to very high levels of pesticides that were beyond regulatory standards. In the present work, four aromatic plants viz., *Lantana camara* L., *Cymbopogon citratus* (DC) Stapf, *Cymbopogon martinii* (Roxb) Wats., *Mentha piperita* L. were studied as biocontrol properties. Each extracts showed unique bioactive potential, and together they demonstrated that natural products can successfully suppress pest growth and activity without leaving harmful residues. This study helps to contribute to develop a natural, sustainable pest management strategy that can reduce dependence on chemical pesticides and also helps to promote healthy agricultural practices.

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