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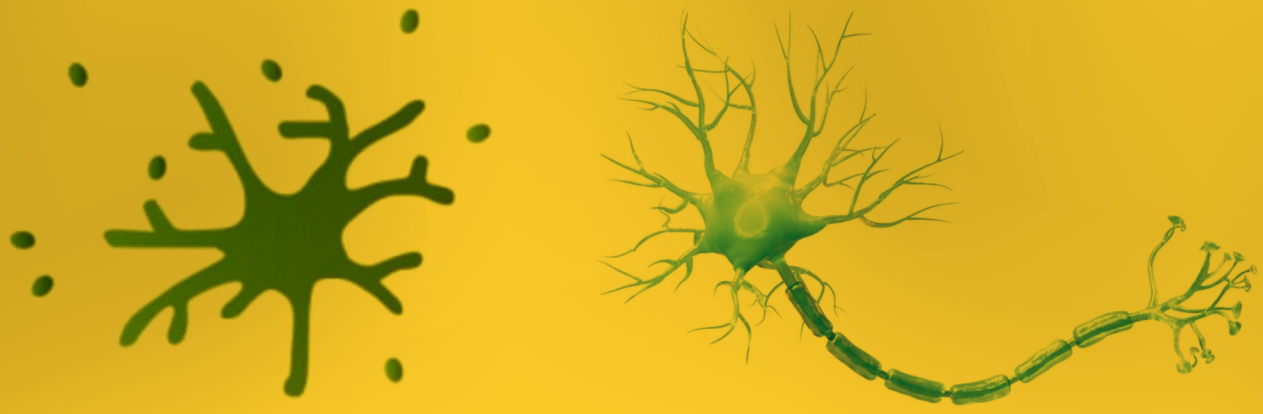

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RESEARCH ARTICLES

Bioscience Biotechnology Research Communications

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Editors Communique

Have we tamed the coronavirus? May be yes,
as pandemics do not die, they can only be faded !

Science and technology has made it possible, in the shortest span of time, it has shown that with firm determination and international cooperation, we can win over the onslaughts of even the worst of the pandemics. COVID-19 is perhaps fading over now, due to our coordinated efforts worldwide. Though we have lost millions, in the two year period, partly due to the mishandling of the viral attacks and somewhat by our own follies and carelessness. Anyway lessons learnt from the past, always make us more stronger and determined. Let us now not relax and work on a better mode, as all is still not well yet. The almost taming of the virus and its cousins have indicated some of the concealed failures, on which we have to focus now. We have to be more vigilant, and even a bit of laxity can spoil the good work done. On societal and governmental parts, utmost care and caution is required on a long term basis.

On behalf of Bioscience Biotechnology Research Communications, we falter at words to express our deep sense of solitude and grief on the catastrophic events of the world wide pandemic, spanning over two years now. We pray for the strength to bear this universal calamity and come up with long lasting fortitude to eradicate it soon.

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Quality publication is one of the ways to keep science alive, and good journals have a leading role to play in shaping science for humanity! As teachers, we have great responsibilities, we have to advocate our students to accomplish and show them the path to test their mettle in hard times to excel, especially in the post COVID 19 era. Science and its advocates will rise more to the occasion and will soon provide succor to the already grief stricken humanity.

Sharique A. Ali, PhD
Editor-in-Chief

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Third Central Incisor as Supplemental Supernumerary Tooth: A Case Report

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ABSTRACT

The presence of supplementary teeth is a frequent developmental abnormality that can cause a variety of clinical issues. Impaction, delayed eruption, ectopic eruption, crowding, etc. are examples of clinical issues. Depending on their morphology, a single extra tooth might be conical, tuberculate, or supplementary. There are two types of multiple extra teeth: syndromic and non-syndromic. Their nature and position following appropriate clinical and radiographic tests determine how it should be managed. This case report describes a patient who has three maxillary central incisors, which are permanent and completely separated. It was determined to be the midline unilateral left supplemental central incisor. In these situations, setting up an appropriate treatment strategy is crucial. A comprehensive clinical and radiographic examination, as well as a detailed history, especially the family history in the event of a systemic anomaly, are required. Its consequences and aesthetic issues can be reduced by following a thorough treatment plan. In these situations, a fixed orthodontic treatment plan that includes the extraction of the supplemental central incisor may be the best course of treatment.

KEY WORDS: SUPERNUMERARY TEETH; CENTRAL INCISOR; SUPPLEMENTAL TEETH; MESIODENS; CROWDING OF TEETH;

INTRODUCTION

Any structure or teeth that are present in excess of the regular dentition are referred to as supernumerary teeth. Teeth may become misaligned as a result of them being impacted or ectopically erupted (Suljkanovic et al, 2021). There are numerous theories that attempt to explain their poorly understood origin. A few significant theories include the theories of hyperactivity, the dichotomy of the tooth bud (tooth germ producing two or more separate units), inheritance, excessive dental lamina growth, atavism (a reversion to a more primitive type of dentition), and that they are just remnants of the Anthropoids, who had more teeth than Homosapiens (Ata-Ali et al, 2014). Gardner syndrome, cleidocranial dysplasia, and cleft lip and palate are frequently linked to an increased frequency of extra teeth. The number, shape, and position of supernumerary teeth determine their classification (Subasioglu et al, 2015).

Depending on number, they may be single or multiple. According to their morphology, a single supernumerary tooth can be conical, tuberculate, odontome, closely resemble the natural tooth, or be supplemental. There are two types of multiple extra teeth: syndromic and non-syndromic (Shetty et al, 2019). The majority of syndromic multiple supernumerary teeth cases are observed in individuals with cleft lip and palate. The morphology of a supplemental tooth is identical to that of the normal neighbouring teeth, showing no anatomical differences. Although they can form anywhere along the dental arches, they are more common in the permanent dentition than in the deciduous dentition, and they are primarily seen in the maxillary anterior region (Tbeishat et al, 2024).

In the deciduous dentition, the prevalence of extra teeth ranges from 0.3% to 0.8%, whereas in the permanent dentition, it ranges from 1.5% to 3.5%. Males are more likely than females to have extra teeth (2:1), and Asian groups are more likely to have them. They may develop at any point in time prior to birth or up to ten years later (Ata-Ali et al, 2014).

Teeth that are supernumerary might remain in their positions for a long time without causing any clinical problems to the dentition. They could become eruptive, remain impacted, seem inverted, or take on an unusual ectopic posture (Gupta and Marwah 2012). The majority of the time, though, they result in local disruptions like midline diastema, crowding and malalignment of the incisors, displacement and rotation

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of the neighbouring teeth, potential dentigerous cyst development, root resorption or dilaceration, and migration into the maxillary sinus or nasal cavity. In the maxillary anterior region, extra teeth can also be an aesthetic concern. In these situations, interceptive orthodontics is crucial in preventing the development of malocclusion (Sarne O et al, 2018). The severity of the malocclusion will undoubtedly be lessened if caught early enough to prevent negative consequences (Männchen et al 2022). This case study describes the unusual occurrence of an additional maxillary central incisor in the midline of a seventeen-year-old male with permanent dentition.

Table 1. Details of the extra oral facial profile/parameters of the patient.

View	Parameters	Classification	Figure
Frontal View	Face Type	Mesocephalic	1a
	Symmetry	Fairly symmetrical	
	LAFH:	WNL	
	Lip Competency	Competent	
	Inter-labial Gap	None	
Frontal Smiling View	Incisal Display	90%	1b
	Gingival Display	IDP of posteriors	
	Buccal Corridors	Asymmetrical (Rt. wider)	
	U Midline to Face	M of #11 to Rt. 3mm	
	L Midline to Chin	Coincident	
	Profile Type	Convex	
	Malar Prominence	Flat	
	Naso-Labial Angle	Slightly increased	
	Upper Lip Thickness	WNL	
Profile View	Upper Lip Length	16mm	1c
	Lower Lip Length	44mm	
	Lower Lip Thickness	WNL	
	Mento-Labial Sulcus	Average	
	Chin Morphology	Slightly retruded	
	Throat-Neck Angle	Slightly Obtuse	

Case Report: The Department of Pediatric Dentistry and Orthodontics at King Saud University in Riyadh, Saudi Arabia received a complaint from a seventeen-year-old male Saudi young adult. He was mostly upset about his "crooked teeth," or lacklustre appearance. He had no prior health concerns, based on the patient history. Nonetheless, the dental history did reveal several previous endodontic and restorative operations. Examining the patient's siblings produced no meaningful findings.

Figures 1 and 2 show the intraoral images and the patient profile, respectively. Tables 1 and 2 contain a list of the intraoral and extraoral examination details. Because it resembled the nearby normal central incisors

morphologically, the central incisor that faced the midline was referred to as a supernumerary or supplemental central incisor. A difference in arch length resulted in a minor rotation of the central incisors (Table 2 & Figure 3). Three maxillary central incisors were visible on an orthopantomogram (OPG) (Figure 4). Multiple restored teeth and lower central incisors with endodontic treatment were also seen on the OPG. Thermal and electric pulp tests yielded positive results for all three upper central incisors. Figure 5 displays the specific readings of the different parameters obtained from the lateral cephalogram. The diagnosis and treatment plan was communicated explained in details to the patient and his parents.

Figure 1: a: Frontal view of the patient.; b: Frontal smiling view of the patient.; c: Lateral Profile view of the patient.

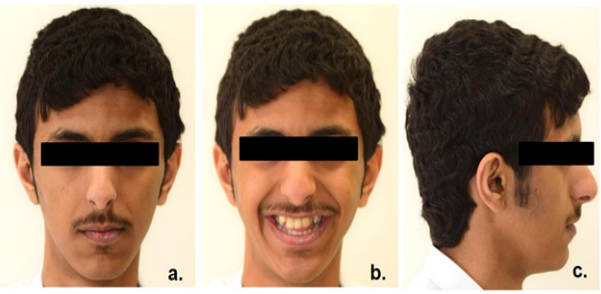


Table 2. Details of the intra oral features/parameters of the patient.

View	Parameters	Classification	Figure
Intraoral Frontal View	Oral Hygiene	Poor	2c
	Soft Tissue	Thin & Inadequate attached gingiva related to L 3-3	
	OB	Open bite #14-#25 of 1-4.5 mm	
	U/L dental midline	L to Lt. 3 mm to M of #11	
	CR/CO shift	None	
	Cross-bite	Edge to edge #16, 4s & 5s	
Intraoral Right View	Over Jet	2 mm	2b
	Canine Classification	½ unit Cl II	
	Molar Classification	Cl I	
Intraoral Left View	Over Jet	2 mm	2e
	Canine Classification	½ unit Cl III	
	Molar Classification	Cl I	
Upper Arch View	Form	Ovoid	2a
	Arch Symmetry	Asymmetrical (A-P)	
	U 3s	displaced B	
	#12	blocked P	
	Supernumerary tooth	b/w centrals	
Lower Arch View	Form	Ovoid	2d
	Arch Symmetry	Asymmetrical (A-P)	
	Teeth	Multiple rotated	
Space Analysis	Upper	-11 mm	-
	Lower	-6 mm	
Bolton Analysis	Mandibular	Excess	-
	Anterior ratio (3-3)	1.8 mm	
	Overall ratio (6-6)	1 mm	

Further radiographic investigations were also carried out in the form of CBCT which revealed normal apex closure for the three central incisors (Figure 6).

DISCUSSION

A third central incisor that is a supernumerary or supplemental tooth is the subject of this extremely uncommon case report.

The majority of extra teeth typically occur in the maxillary midline and are referred to be mesiodens; general dentists are well-versed in treating these types of teeth (Akitomo et al, 2023). The extra or supernumerary tooth in this unusual case report, however, was a fully grown central incisor. Although they have been documented in the literature, bilateral supernumerary/supplemental central incisors are uncommon; even more uncommon are occurrences of solitary supernumerary/supplemental central incisors. In fact, there have only been five occurrences documented in the literature to date (Kumar et al, 2012).

The majority of supplemental teeth are still unerupted, and this has been linked to a number of pathological problems, including ankylosis, expanded follicular space, dentigerous cyst formation, dental pulp necrosis, pulp canal obliteration, and root resorption. Common issues include permanent tooth rotations, diastema development, and disturbances in eruption (Chalakkal et al, 2018). Because they are typically aberrant in size and shape, most erupted supernumerary teeth are excised for cosmetic reasons. On the other hand, Holtzman L, reports an example of a preserved erupted conical supernumerary tooth (Holtzman 1998).

Figure 2: a: Occlusal view of the upper arch.; b: Right lateral view of the upper/lower teeth in occlusion.; c: Frontal view of the upper/lower teeth in occlusion.; d: Occlusal view of the lower arch.; e: Left lateral view of the upper/lower teeth in occlusion.

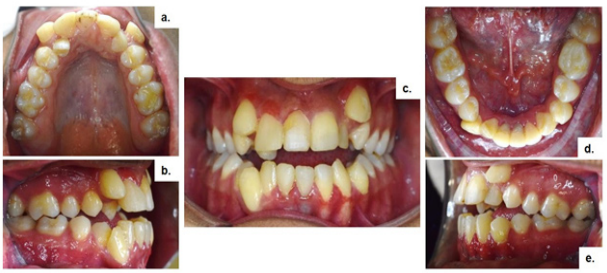


Figure 3: Casts fabricated and used for space analysis of the patients upper and lower arches.

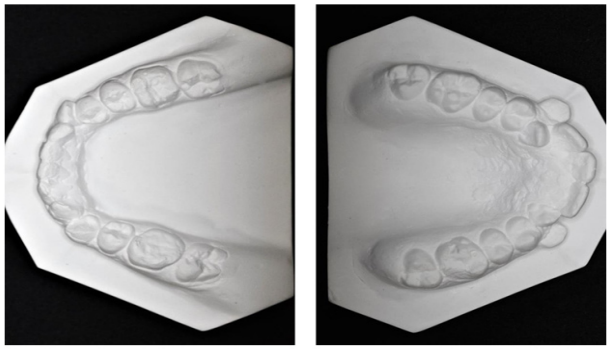
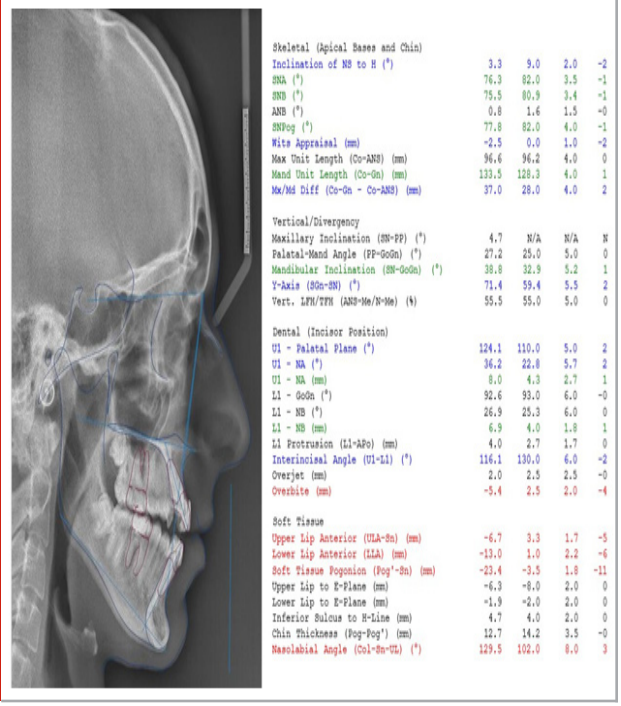


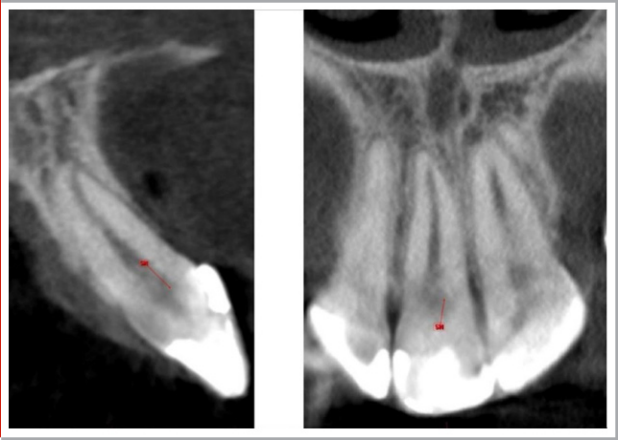
Figure 4: OPG of the patient showing permanent dentition 7-7 with the supernumerary, all 8s are present. Dilacerated roots of #22, #34 & #44. WNL Asymmetrical condyles (left side smaller).

Figure 5. Lateral Cephalogram of the patient with all the measurements.



Skeletal (Apical Bases and Chin)				
Inclination of NS to H (°)	3.3	9.0	2.0	-2
SNA (°)	76.3	82.0	3.5	-1
SNB (°)	75.5	80.9	3.4	-1
ANB (°)	0.8	1.6	1.5	-0
SNPog (°)	77.8	82.0	4.0	-1
Wits Appraisal (mm)	-2.5	0.0	1.0	-2
Max Unit Length (Co-ANS) (mm)	96.6	96.2	4.0	0
Mand Unit Length (Co-Gn) (mm)	133.5	128.3	4.0	1
Mx/Md Diff (Co-Gn - Co-ANS) (mm)	37.0	28.0	4.0	2
Vertical/Divergency				
Maxillary Inclination (SN-PP) (°)	4.7	N/A	N/A	N
Palatal-Mand Angle (PP-GoGn) (°)	27.2	25.0	5.0	0
Mandibular Inclination (SN-GoGn) (°)	38.8	32.9	5.2	1
Y-Axis (SGo-SN) (°)	71.4	59.4	5.5	2
Vert. LFH/TFH (ANS-Me/N-Me) (%)	55.5	55.0	5.0	0
Dental (Incisor Position)				
U1 - Palatal Plane (°)	124.1	110.0	5.0	2
U1 - NA (°)	36.2	22.8	5.7	2
U1 - NA (mm)	8.0	4.3	2.7	1
L1 - GoGn (°)	92.6	93.0	6.0	-0
L1 - NB (°)	26.9	25.3	6.0	0
L1 - NB (mm)	6.9	4.0	1.8	1
L1 Protrusion (L1-APo) (mm)	4.0	2.7	1.7	0
Interincisal Angle (U1-L1) (°)	116.1	130.0	6.0	-2
Overjet (mm)	2.0	2.5	2.5	-0
Overbite (mm)	-5.4	2.5	2.0	-4
Soft Tissue				
Upper Lip Anterior (ULA-Sn) (mm)	-6.7	3.3	1.7	-5
Lower Lip Anterior (LLA) (mm)	-13.0	1.0	2.2	-6
Soft Tissue Pogonion (Pog'-Sn) (mm)	-23.4	-3.5	1.8	-11
Upper Lip to E-Plane (mm)	-6.3	-8.0	2.0	0
Lower Lip to E-Plane (mm)	-1.9	-2.0	2.0	0
Inferior Sulcus to H-Line (mm)	4.7	4.0	2.0	0
Chin Thickness (Pog-Pog') (mm)	12.7	14.2	3.5	-0
Nasolabial Angle (Col-Sn-UL) (°)	129.5	102.0	8.0	3

Figure 6. CBCT images of the supernumerary/supplemental permanent central incisor tooth.



In men, supernumerary/supplemental cases are marginally more prevalent (Mathew et al, 2023). The male case in ours is 17 years old young adult. In between the supplementary

central incisor are two permanent central incisors. The lateral incisors erupted palatally, while the canines erupted buccally. All of the lower incisors had erupted, but the lower arch teeth were largely normal. Since the supernumerary/ supplemental tooth in this case study is likewise positioned in the upper jaw, the incidence of hyperdontia in the upper jaw is eight times higher than in the lower jaw (Mathew et al, 2023).

Most supernumerary teeth are unable to erupt normally, and when they do, it's usually ectopically, emerging alongside regular teeth. In our instance, however, an extra permanent central incisor tooth (supernumerary/supplemental) erupted in the upper arch precisely between the two regular permanent central incisor teeth, taking its position within the arch without becoming ectopic (Ata-Ali et al, 2014; Kumar et al, 2012). Regarding the extra permanent tooth, its future impact on the dental arch is uncertain as it has not yet broken through. Removal of a supernumerary premolar should be done to reduce crowding and/or prevent occlusal discrepancies if it is erupting or is crowded after eruption, according to Khalaf K et al, 2018.

To restore the natural appearance and functionality in this instance, it was determined to extract the erupted supernumerary/supplemental central incisor. The rehabilitation was to begin with the standard protocol of periodontal/restorative assessment and management before the extraction of the supernumerary/supplemental tooth, followed by fixed orthodontic treatment in order to establish a good occlusion, because the patient came to the clinic at an age when all of his permanent teeth had fully erupted and he had a full set of dentition (Suljkanovic et al, 2021).

Before a final diagnosis and treatment plan are developed, it is imperative to count and detect supernumerary/ supplemental teeth both clinically and radiographically. A good treatment strategy requires accurate identification of every tooth both clinically and radiographically. Creating the perfect treatment strategy for every patient with extra or supernumerary teeth may be challenging (Kumar et al, 2012; Suljkanovic et al, 2021).

However, an attempt can undoubtedly be made. Depending on the type, quantity, and location of any extra teeth—whether impacted or erupted—as well as any accompanying pathology and how it affects the neighboring teeth, treatment of any extra teeth should be approached carefully, taking into account issues with oral cleanliness and aesthetics (Gupta and Marwah 2012). The course of treatment might range from simple elimination of unnecessary teeth to extraction and orthodontic correction to create a healthy occlusion. Although there isn't a set course of treatment for this illness, most experts advise extraction. This is, in fact, the methodology that is taught in undergraduate and graduate textbooks (Baxi et al, 2023).

Common issues include permanent tooth rotations, diastema development, and disturbances in eruption. Finding and identifying issues related to additional or supernumerary teeth is the initial step in the management process. The teeth are routinely pulled, which usually requires surgery, if issues are present (Ahammed et al, 2021). Early extraction of supernumerary/supplemental teeth that produce incisor impaction may reduce loss of eruptive potential, space loss, and centerline displacement. Early extraction of the supplemental or supernumerary tooth that is causing the rotation of the unerupted incisors can lead to self-correction and proper alignment, even in cases when the rotation is severe (Acharya 2015).

The possibility that early removal will interfere with the development of neighboring roots is the biggest worry. It takes anywhere from six months to three years for the unerupted tooth to erupt following the extraction of supernumerary teeth. On the other hand, fully erupted supernumerary/supplemental maxillary central incisors may cause a significant crowding of the upper arch teeth, pushing the lateral incisors toward the palate and dislodging the canines to the buccal region, leading to malocclusion. Managing the gap left after the supernumerary or supplemental tooth is extracted presents one of the biggest hurdles in these circumstances (Meighani and Pakdaman 2010).

CONCLUSION

When a patient has extra or supernumerary maxillary central incisors, the practitioner should be made aware of the possible risks. Any patient who has extra or supernumerary maxillary central incisors should undergo a comprehensive evaluation. During a patient's initial assessment, a comprehensive clinical examination should always be carried out in addition to any recommended radiographic imaging. CBCT may be used when additional information is required for a precise diagnosis and treatment plan. Early detection and therapy, essential components of preventive dentistry, can help prevent orthodontic issues and dental pathology related to supernumerary/supplemental maxillary central incisors. This could have a significant positive impact on the quality of life for young patients. Depending on the kind, quantity, and placement of the teeth as well as any potential issues, especially those involving aesthetics, a thorough treatment plan should include both surgical extraction and orthodontic correction followed by long-term retention.

Patient Consent Declaration: The authors certify that they have all required patient consent paperwork in their possession. The consent form for the patient(s) and/or their parents allows the publication of the patient(s)' photos and other clinical data in the journal. The patients are aware that although every attempt will be made to hide their identity, anonymity cannot be guaranteed and that their names and initials will not be published.

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Impact of some Natural Resources on Biological Aspect of Tuta absoluta M. Under Laboratory and Green House Conditions with Reference to its Enzymatic Activity

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ABSTRACT

This study was targeted to test four vegetable oils namely (*Ruta chalepensis, Azadirachta indica, Simmondsia chinensis, Nigella sativa* K.L.) against tomato leaf miner *Tuta absoluta* under laboratory and field conditions, in addition to record its effect on their enzymatic activities. The data illustrated that *N. sativa* and *R. chalapensis* caused the highest reduction in eggs hatchability, mortality larval instars and moderate effect in larval penetration under laboratory condition. On other side, the field evaluation was noticed that the most effective one was *S. chinensis* followed by *A. indica* and *R. chalepensis* which recorded reduction in insect infestation reach to ≥ 72 % on sprayed green part. On other hand, the least fruit infestation was recorded in case of treatments by R. chalepensis followed by *S. chinensis, A. indica* and *N. sativa*. The enzymatic analysis was given spot light on the reason of the variation in the effect among the tested oils toward 4th larval instars. Data was showed that the compounds reduced the enzymatic activities, which might suggest a poor defense mechanism in the detoxification of the used oils.

KEY WORDS: *TUTA ABSOLUTA* - VEGETABLE OILS - ENZYMATIC ACTIVITIES- EGGS- LARVAL MORTALITY- CROP PRODUCTION- FRUIT INFESTATION,

INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill, is the most important and lucrative vegetable crop around the world which is planted in both outdoors and under green houses. The tomato crop yield has been faced different factors leading to reduce their productivity including pests and diseases (Materu et al., 2016 and Kandil et al., 2020). The tomato yield productivity reduced up to 100% in different governorate of Egypt due to the invasion with a newly dangerous insect pest namely tomato leaf minor *Tuta absoluta* (Meyrick) (Moussa et al., 2013; Soares et al., 2019; Mansour & Biondi, 2021 and Ahmed et al., 2022).

In early infestation, newly emerged neonates penetrate tomato leaf into the mesophyll layer and feed between the lower and upper surfaces of the leaf to form small and transparent mines. The larvae attack all other parts of the tomato plant except only the root (Kandil et al., 2020 and Ahmed et al., 2022). The application of chemical insecticides is the most effective method for management *T. absoluta*. However, such strategy has a number of disadvantages including development of insect resistance towards conventional insecticides, environmental pollution, and potential toxicity to non-target organisms (Maneno et al., 2015; Abouelfadal, 2016, Campolo et al., 2018 and Ahmed et al., 2022).

Along the late decades all over the world, a variety of botanical extracts as alternatives to chemical insecticides for controlling different insect species have been examined (Campolo et al., 2018; Fergani and Yehia., 2020). The insecticidal activities of various plant species against T. absoluta have been proven (Nadia et al., 2014; Moawad et al. 2013 and Esther et al., 2019 Al-Solami, 2021; Erbas and Altuntas, 2021; Moawad and Ebadah 2022 and Moawad et al., 2022).

The ability of plant extracts to reduce or suppress antioxidant and detoxifying enzymes activities may improve the insecticidal efficacy of the botanical extract-based formulation, as well as exploited as synergistic ingredient to enhance the efficacy of other insecticides (Campolo et al., 2018). Therefore, estimation the biochemical effects of T. absoluta toward insecticidal plant extracts are critical

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to develop new options for their control (Ayil-Gutierrez et al., 2018). Additionally, estimation of such enzymes could help to propose or find new biological control agents. The present study aims to evaluate the efficiency of some natural resource oils against tomato leaf miner *T. absoluta* for developing the management strategy for such pest. In addition, the biochemical changes in some antioxidant and detoxifying enzymes will be investigated.

MATERIALS AND METHODS

The experiments were carried out to test four types of natural oils namely (*Ruta chalepensis, Azadirachta indica, Simmondsia chinensis, Nigella sativa* K.L.) which were obtained from Luna Company Egypt. The preparation of tested oils concentration 10% was followed methods (Katoune et al., 2011 and Moawad and Sadek, 2018). 10% oil concentration was done by dissolved 10 ml of tested oil in 80 ml distilled water + 8 ml Arabic gum (20%) + 2 ml tween (20%) with add two drop from glycerin.

Laboratory experiments: Insect culture: To test oils on immature stages of *T. absoluta* (eggs and larvae) the infested tomato leaves were collected from field to start rearing. The larvae were reared and maintained on tomato leaves, cultivated in plastic pots inside a glass cage (50x 50x100cm3). The culture was provided by infested tomato leaves harboring *T. absoluta* pre-imagine stages collected from the field for isolation of eggs or outer larvae and pupae to maintain it. Newly emerged adults were collected and transferred to another glass cage (50x50x100 cm3) containing untreated plastic pots of tomato. The experiments were carried out on the 1st generation of tomato leaf miner.

Treatment of egg stage: Leaves from the maintained culture were examined under Stereo-binocular to isolate deposited eggs by fine brush and keeping them in a Petri-dish. Eggs of one day old were used in the experiments. The tested oils was sprayed on eggs and let till dry. Each test was used 30 eggs and it was replicated five times. Percentages of reduction in eggs hatchability were calculated as follows: Reduction of eggs hatchability % = a – b/a x 100 Where; a= number of eggs hatched in the control, b= number of eggs hatched in the treatment.

Treatment of larval stages: Couples of males and females were placed in glass tubes (10 cm.) for egg deposition and for facilitating obtain of 1st instar larvae. While other larval stages (3rd and 4th instar) were collected directly from the infested tomato leaves. In case of exposure 1st instar to treatment was investigated leaves daily to calculated penetration percentage and follow up till record pupation %.On other target the treatment of last larval stage (3rd and 4th) was observed and recorded their mortality and pupation %. Mortality % in all treatments was corrected by Abbott's formula (Abbott, 1925).

Mortality % = (T - C) / (100 – C) x 100, Where: T=Mortality in the treatment C= Mortality in the control

Green house experiments: The present study was carried out in a plastic green house (9 x 40 m2) in reclaimed desert sandy soil in Nubaria region, Egypt and cultivated with tomato variety at winter plantation. The green house area 360 m2 was randomly divided into six experimental blocks, each block (5 rows, 7 plants/ row i.e. 35 plants/block) was specified for each treatment and two block were specified for the control +additive and control (without any treatment). The whole tested area was followed normal agricultural practices. Each block was divided to three replicates. Each one was sprayed twice interval time by tested oil.

The first spraying was done after one month while second one was done after two months of tomato plantation. Tested oils were sprayed by using a manual sprayer (10 litter / plot). To evaluate the effect of tested oils on population of T. absoluta the randomly sample were collected from each replicate before spraying followed by subsequently samples after spraying (5,7,10,and 15 days). Examination of tomato leaflets were done under stereomicroscope to count number of deposited eggs and tunnel were targeted to calculate the reduction percentage in insect population. The reduction percentage of population density of T. absoluta was calculated according Henderson and Tilton (1955) equation as follows:

R % = 1 - (no. of individuals in control before treatment X no. of individuals in treatment after treatment / no. of individuals in control after treatment X no. of individuals in treatment before treatment) X100.To evaluate the effect of treatments on crop production and infestation percentage of tomato fruits were done once for first spray (by let 100 plant without 2nd spraying) and other one for 2nd spray by pick up the whole fruits to investigate and weight.

Enzyme assays: Polyphenol oxidase: Polyphenol oxidase (PPO) activity was conducted using L-3,4-dihydroxyphenylalanine (DOPA) as substrate according to Leonard (1971) and modified by Taleh et al. (2014) . The reaction mixture was contained in 1.0 ml: 100 mM potassium phosphate buffer, pH 7.0, 10 mM DOPA and enzyme crude extract ranged from 10.0-50.0 µl. The increase in the absorbance was recorded for 5 min at 470 nm. One unit of PPO activity was defined as the amount of enzyme that cause changes of 0.1 O.D./min under standard assay conditions.

Peroxidase: Peroxidase activity (PO) activity was determined according to Lee (1973) and modified by Aydinz and Kadioglu (2001) using guaiacol as substrate. The reaction mixture was contained in 1.0 ml: 100 mM sodium acetate buffer, pH 5.6, 20 mM guaiacol, 30 mM hydrogen peroxide (H2O2) and enzyme crude extract ranged from 5.0-20.0 µl. The increase in the absorbance was recorded for 3 min at 470 nm. One unit of PO activity was defined as the amount of enzyme that cause changes of 1.0 O.D./ min under standard assay conditions.

Catalase: Catalase (CAT) activity was routinely assayed according to the method described by Aebi (1984). The reaction mixture contained in 2 ml; 20 mM H2O2 and 50

mM phosphate buffer, pH 7.0. The reaction was started after addition of suitable amount of enzyme solution (0.15 mg protein). The decomposition of H2O2 was followed as a decline in absorbance at 240 nm for 1 min at 27oC. One unit of CAT activity was defined as the amount of enzyme capable of catalyzing the decomposition of 1 µmol of H2O2/min at 27oC using an extension coefficient of 43.6 M-1 cm-1.

Carboxylesterase: Carboxylesterase (CaE) activity was measured using P-NPA as substrate according to Galliard and Dennis (1974). The reaction mixture contained in 1.0 ml: 2 mM of P-NPA and 100 mM phosphate buffer, pH 7.5. The change in absorbance was recorded at 407 nm for 10 min. One unit of CaE activity was defined as µmoles of P-nitrophenol produced per hour under standard assay conditions.

Table 1. Effect of 10% concentration of tested natural plant oils on eggs hatchability and embryonic development of *T. absoluta*.

Treatment	Reduction in eggs hatching %	Embryonic development %
Ruta chalepensis	86.7	93.3
Simmondsia chinensis	78.8	36.7
Azadirachta indica	65.9	95.8
Nigella sativa	94	0.0
Control+additive	5.9	100
control	0.0	100

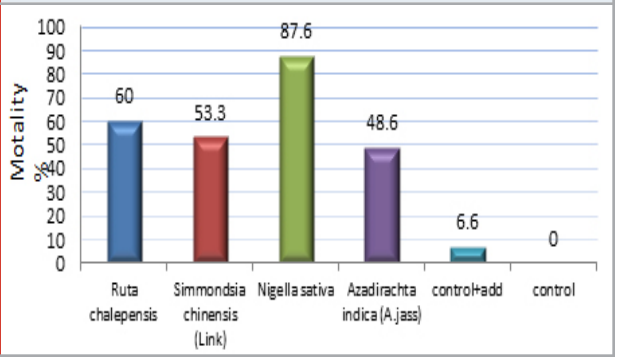
Table 2. Effect of treatment leaflets by 10% concentration of tested natural plant oils on 1st larval stage penetration and pupation percentage of T. absoluta.

Treatment	Penetration %	Pupation %
Ruta chalepensis	60	22.2
Simmondsia chinensis	53.3	4.5
Azadirachta indica	48.6	0.0
Nigella sativa	77.6	11
Control+additive	92.6	87
control	100	96

Acetylcholinesterase: Acetylcholinesterase (AChE) activity was measured using AcSChI as substrate according to Ellman et al. (1961). The reaction mixture contained in 1ml: 60 mM Tris-HCl buffer, pH 8.5, 1 mM AcSChI, 1 mM DTNB. The reaction mixtures were incubated at 37oC and the increase in the absorbance was recorded at 412 nm. One unit of AChE activity was defined as the amount of enzyme that catalyses hydrolysis 1µmol of substrate per hour under standard assay conditions.

Acid phosphatase: Acid phosphatase (AcP) activity was measured using P-NPP as substrate for according to the method described by Dinan et al., (1983). The reaction mixture contained in 0.5 ml: 2 mM of P-NPP and 100 mM acetate buffer, pH 5.5. The reaction mixture was incubated at 37 for 30 min and terminated by adding of 1.0 ml of 0.1M NaOH. The increase in the absorbance was recorded at 410 nm. One unit of AcP activity was defined as µmoles of P-nitrophenol produced per hour under standard assay conditions.

Figure 1: Effect of tested oils on mortality percentage of 3rd and 4th instar larvae of T. absoluta.



Protein determination: Protein contents were determined according to Bradford (1976) using bovine serum albumin as a standard.

Statistical analysis: All data were subjected to analysis of variance (ANOVA) and the means were compared by LSD test at 0.05 levels, using SAS computer program (SAS, 2009).

RESULTS

Effect of tested oils on different stages of T. absoluta under laboratory condition

On Eggs stage: Table1 illustrated that N. sativa and R. chalapensis caused the highest reduction in eggs hatchability reached to 94 and 86.7% but embryonic development was varied from 0.0 to 93.3%. While S.chinensis and A. indica were recorded 78.8 and 65.9% reduction in eggs hatchability and 36.7 and 95.8% embryonic development compare to control 0.0%. The result indicated that the most of tested oils didn't cause direct toxicity its effect might be attributed to endocrine disturbance; except in case of treatment by N. sativa which had toxicity effect.

On larval instars: Table 2 illustrated that the tested oils were moderately effect on percentage of 1st larval penetration but the most of them failed to complete cycle till pupation. Treatments by A. indica were recorded 48.6 % larval penetration and 0.0% pupation. On other treatments by N. sativa, S.chinensis and R. chalepensis were recorded penetration percentage ranged between (48.6 to 60%) and pupation % (0.0 to 22.2%). While Treatment 2nd, 3rd and 4th larval instar by tested oils were recorded mortality percentage reached to 87.6% in case of treatment by N.

sativa (Fig.1). The remaining tested oils were recorded mortality % more than 48% and not more 60% compare to control + additives were record 6.6% mortality %.

II- Effect of tested oils on control T. absoluta infestation under field condition

On Deposited eggs: The table3 cleared that all of tested oils were recorded reduction percentage in eggs deposition compare to control. the highest reduction percentage in case of treatment by S. chinensis which reached to 70.6, followed by N. sativa, R. chaepensis and A. indica which ranged from 58 to 48%.

On infestation rate of green part of tomato: Data in table 4 gave indication on reduction percentage of Tuta infestation due to spray leaflets by tested oils. The most effective one was S. chinensis followed by A. indica and R. chalepensis which recorded reduction % in insect infestation reach to 78.4, 72.4 and 71.4 compare to control + add were recorded 13.8%.

Table 3. Effect of tested oils on percentage reduction in eggs deposition of *T. absoluta* at different time intervals under greenhouse conditions

Test oils / Date Of Examination	*Ruta chalepensis*		*Simmondsia chinensis*		*Azadirachta indica*		*Nigella sativa*		Control+ additive		control	
	Deposited Eggs / 50 leaflets											
	number	reduction%	number	reduction%	number	reduction%	number	reduction%	number	reduction%	number	reduction%
For 1st spray												
pre-treatment	49	....	61	....	52	....	64	....	65	...	47	...
5th day	25	44.2	32	42.7	40	15.9	48	34.9	51	4.4	43	...
7th days	22	42.9	12	75.01	25	38.9	31	58	32	37.5	37	....
10 th days	36	41.5	23	69.9	26	60.2	19	74.3	48	41.2	59	....
Two weeks	34	61.6	14	87.3	43	54.3	16	78.3	69	41.3	85	....
General mean	33.2	47.6±4.7	28.4	68.7±9.4	37.2	42.3±9.9	35.6	63.8±9.9	53	31.1±8.9	54.2	....
Statistical analysis	L.S.D.0.05=23.5 L.S.D.0.01=32.54											
2nd spray												
pre-treatment	106	...	144	...	94	...	159	....	112	....	98	....
5th day	66	53.4	62	67.8	71	43.5	84	60.5	125	16.5	131	....
7th days	44	65.5	46	73.5	43	62.01	76	60.3	111	17.8	118	....
10 th days	42	57.7	40	70.4	49	44.5	70	53.1	104	1.1	92	....
Two weeks	57	48.3	44	70.7	56	42.8	63	61.9	122	4.7	102	....
General mean	63	56.3±3.6	67.2	70.6±1.2	62.6	48.2±4.6	90.4	58.9±1.98	114.8	9.98±4.2	108.2	
Statistical analysis	L.S.D.0.05=8.91 L.S.D.0.01=12.54											

Table 4. Effect of tested oils on the reduction percentage of *T. absoluta* infestation at different time intervals under greenhouse conditions

Test oils / Date Of Examination	*Ruta chalepensis*		*Simmondsia chinensis*		*Azadirachta indica*		*Nigella sativa*		Control+ additive		control	
	No. of larvae /50 leaflets											
	Alive larvae	reduction%	Alive larvae	reduction%	Alive larvae	reduction%	Alive larvae	reduction%	Alive larvae	reduction%	Alive larvae	reduction%
For 1st spray												
pre-treatment	93	....	76	.....	69	....	75	...	91	...	84	...
5th day	77	20.1	36	54.3	44	38.4	47	24.6	86	8.8	87	...
7th days	64	39.8	18	79.3	52	30.1	22	64.7	89	14.4	96	....
10 th days	18	66.8	7	84.2	15	62.7	9	85.6	37		49	....
Two weeks	24	34.3	10	66.5	13	52.1	12	80.7	29		33	....
General mean	45.8	40.2±9.8	29.4	71.1±6.7	38.6	45.8±13.8	33	63.9±13.8	60.3	8.9±2.01	69.8	....
Statistical analysis												L.S.D.0.05=23.6 L.S.D.0.01=32.7
2nd spray												
pre-treatment	102	...	89	...	65	....	94	....	54	...	80	....
5th day	43	58.9	35	61.6	27	59.5	41	57.5	48	13.3	82	....
7th days	31	68.0	8	90.5	14	77.3	32	64.2	39	23.9	76	....
10 th days	17	80.4	17	77.5	12	78.3	23	71.2	42	8.5	68	....
Two weeks	20	78.5	13	83.9	15	74.7	15	28.5	54	9.6	73	....
General mean	42.6	71.4±4.99	32.4	78.4±6.2	26.6	72.4±4.4	41	55.4±9.4	47.4	13.8±3.5	75.8	....
Statistical analysis												L.S.D.0.05=16.13 L.S.D.0.01=22.36

Figure 2: Antioxidant enzymes activity in 4th instar larvae of T. absoluta before and after treatment with tested botanical oils. Each result represents the average of three separate experiments ± SE. (a) CAT, (b) PPO and (c) PO.

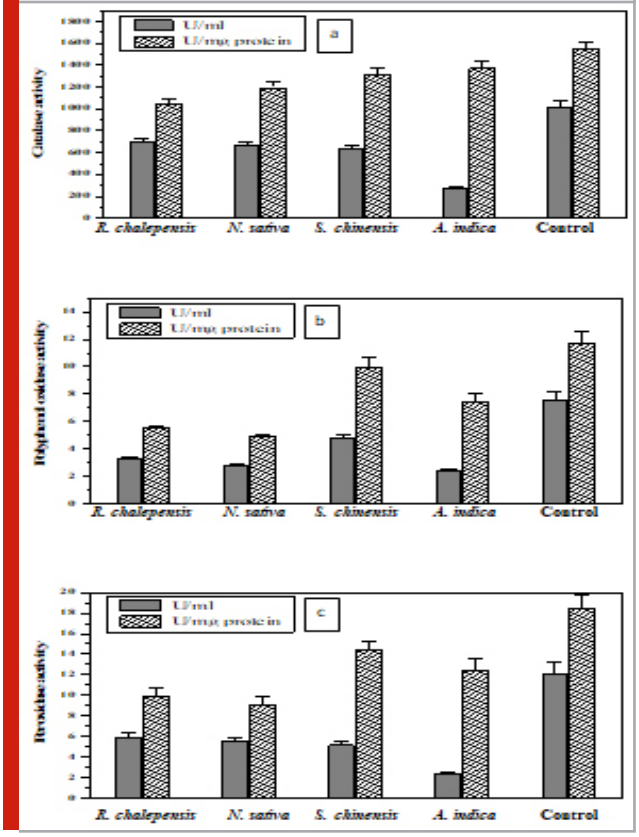


Figure 3: Detoxifying enzymes activity in 4th instar larvae of T. absoluta before and after treatment with tested botanical oils. Each result represents the average of three separate experiments ± SE. (a) CaE, (b) AChE and (c) AcP

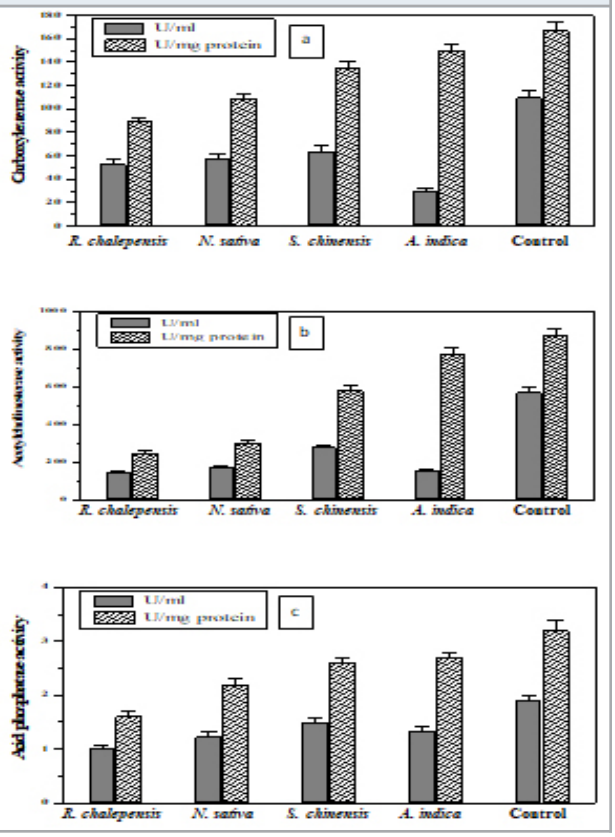


Table 5. Effect of different treatments on the fruit infestation and crop production

Treatment	Fruits Infestation %	Mean of crop weight/kg/plot/ 35 plant (X±S.E)
Ruta chalepensis	14.2	13.6±1.01^{cc}.
Simmondsia chinensis	25.8	15.8±0.5^{cc}.
Azadirachta indica	36.7	12.7±1.1^{cb}.
Nigella sativa	36.7	15.3±0.74^{cc}.
Control+additive	53.1	10.5±0.38^{aa}.
control	71.6	8.9±0.7^{a}
Statistical analysis	L.S.D.0.05=2.03 L.S.D.0.01=2.8	

On fruit infestation and crop production: The rate of fruit infestation in all treatment were recorded ratio less than control or control + add as described in Table 5. the least fruit infestation was recorded in case of treatments by R. chalepensis followed by S. chinensis, A.indica and N. sativa which recorded infestation ranged between 14.2 to 36.7% compare to control were recorded 71,6% infestation. On other view the crop production was affected and recoded highly significance variation between treatments and control (Table 5). The crop weight/kg//plot/35 plot were arranged descending as follow: S.chinensis > N.sativa> R.chalepensis > A. indica which were recorded (15.8, 15.3, 13.6 and 12.7) compared to contol+add or control were recorded 10.5 and 8.9 mean crop weight/35 plant.

Effects of botanical extracts on enzymatic activities

Antioxidant enzymes Catalase: A variation in CAT activity of 4th instar larvae of tomato leaf miner has been demonstrated before and after treatment with tested botanical oils. CAT activity ranged from 275-1022 U/ml with specific activity ranged from 1047-1556 U/mg protein (Fig.2a).

Polyphenol oxidase: In the 4th instar larvae of tomato leaf miner, PPO activity has been determined before and after treatment with tested botanical oils. The activity ranged from 2.5-7.6 U/ml with specific activity ranged from 5.0-11.7 U/mg protein (Fig.2b).

Peroxidase: A variation in PO activity has been detected in 4th instar larvae of T. absoluta. The PO activity ranged from 2.5-12.2 U/ml with specific activity 9.14-18.5 U/mg

protein (Fig.2c). The results showed that R. chalepensis and N. sativa had the most effect on the activity of measured antioxidant enzymes.

Detoxifying enzymes: Carboxylesterase: The activity of carboxylesterase (CaE) was determined in 4th instar larvae of tomato leaf miner before and after treatment with tested botanical oils. The CaE activity ranged from 30-110 U/ml with specific activity 89.5-167 U/mg protein (Fig.3a).

Acetylcholinesterase: A variation in AChE activity of 4th instar larvae of tomato leaf miner has been demonstrated before and after treatment with tested botanical oils. AChE activity ranged from 275-1022 U/ml with specific activity ranged from 146-569 U/mg protein (Fig.3b).

Acid phosphatase: In the 4th instar larvae of T. absoluta, AP activity has been determined before and after treatment with tested botanical oils. The activity ranged from 1.0-1.9 U/ml with specific activity ranged from 1.6-3.2 U/mg protein (Fig.3c). *R. chalepensis* and *N. sativa* exhibited the greater inhibitory effect on the activity of the tested detoxifying enzymes.

DISCUSSION

The present data was cleared that vegetable oils might be used in decrease population of tomato leaf miner to gain good fruits quality production. The laboratory evaluation was made focus on the effect of tested oils on egg and different larval stages which indicated to its hormonal and toxicity effect toward eggs and larval stage. The tested plant oils were also investigated on the activities of two groups of enzymes, antioxidant and detoxifying enzymes. Data was showed that the compounds reduced the enzymes activity, which might suggest a poor defence mechanism in the detoxification of the used oils.

CAT worked in concert to reduce the oxidative stress by detoxifying O_2- to molecular O_2 and H_2O, also PO acted as H_2O_2 scavenger enzyme. CAT can remove H_2O_2 only at high cellular level and is inefficient for scavenging H_2O_2 at low concentration. However, PO acts as a scavenger under all conditions (Mathews et al., 1997 and Jia et al., 2011).

Insects consume plant phenolic compounds which are toxic if ingested at high amounts. Insects have the ability for detoxifying these compounds. PPO has important role in insect's immunity mechanisms (Wu et al., 2015 and Mohamed et al., 2022). PPO has major role for detoxifying the toxicity of plant pro-oxidant allelochemicals, so it can be interpreted that reduced PPO activity in the treated *T. absoluta* resulted in the death of the larvae.

CaEs are vital detoxifying enzymes which hydrolyzes the esteric bond in synthetic chemicals. The response decreases of CaE enzymes to botanical extracts were concurrence with Mojarab-Mahboubkar et al., 2015 and Abdel-Razi, 2018 revealed a decreased amount of esterases. AchE has a key role in neurotransmission by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides (Mohamed et al., 2020 and 2021).

The essential oils exhibited a neurotoxic action resulting in spasms, lack of mechanical coordination and tremors (Abdellaoui et al., 2018). Several previous studies recorded that rapid action of essential oils against pests is an indicative of neurotoxic actions. Bessette et al., 2013 reported that in direct contact, essential oils can penetrate through insect's cuticle and contact the nerve endings, and cause neurotoxic activity and rapid death. The neurotoxic modes of action on insects are mainly related to AChE levels. Many reports have demonstrated the interference of essential oils or its constituents with AChE enzyme activity in insects (Yeom et al. 2013).

AcP is hydrolytic enzyme, which hydrolyze phosphomonoesters under acidic conditions. Changes in AcP activities after treatments indicate that changing the physiological balance of the midgut might affect these enzymes (Ayil-Gutiérrez et al. 2018).Many researchers searched on the effect of botanical materials on control of *T. absoluta*. Nadia et al., 2014 reported that application of four concentrations of neem (*Azadirachta indica*) seeds ethanolic extract and Jatropha (*Jatropha curcas*) seeds petroleum ether extract on young larvae of *T. absoluta* resulted in larval mortalities that ranged between 33%-46.7% and 23.5%-48.5%, respectively, obtained after 24 h. Also, higher larval mortalities, up to 100%, were obtained with the two extracts after 4 d of treatments.

Esther et al., (2019) tested four plant extract (*Commiphora swynnertonii, Synadenium glaucescens* and *Allium sativum*) and found that all plant extracts were effective and controlled adult *T. absoluta* under laboratory condition. While, Commiphora extracts were highly effective and controlled *T. absoluta* in screen house. Foliar application reduced *T. absoluta* population, improved quality and yield of tomato. The results confirmed that inhibition in the enzymes may be the reason explained why *N. sativa* and *R. chalapensis* showed higher mortality than *S.chinensis* and *A. indica*. The mechanism of resistance of *T. absoluta* toward natural products is critical to develop new options for their control. Additionally, an analysis of this mechanism could help to propose or find new biological targets.

Conflict od Interest: Authors declare no conflict of interest

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Data Availability: Data are available with the corresponding author on reasonable request

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Applications of Nano Formulation: New Innovation in Improving Drug Delivery

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ABSTRACT

ANanoparticles are a revolutionary medication delivery technology, as we all know. They have several positive impacts, such as the drug's efficacy and safety. We enumerate their efficaciousness during drug distribution in this review. One of the methods for more precisely delivering pharmacological substances to the intended tissue while lowering the total dosage and possible harmful side effects is drug nanoformulation. They may function as carriers of various active medicinal ingredients into a particularly body regions, or they may be therapeutic agents in and of themselves. As a truly multidisciplinary field of study, nanotechnology has benefited greatly from the contributions of chemists, physicists, biologists, and pharmaceutical scientists in the development of novel therapeutic and diagnostic approaches. The application of nanotechnology has advanced non-invasive imaging, nutraceutical delivery, cancer and HIV/AIDS treatment, and more. There are many benefits to using micro and nanoparticles in biomedicine, particularly when it comes to drug delivery, over traditional methods which include improved drug delivery, high-performance properties of the product, using less costly drug concentrations in the delivery systems, extending the drug's bioactivity by shielding it from environmental effects in biological media, and more effective treatment with fewer side effects.

KEY WORDS: NANOPARTICLES, LIPOSOMES, SURFACE AREA, SHAPE, NANO FORMULATION, NANOFORMULATIONS IMPROVING DRUG DELIVERY.

INTRODUCTION

Polymeric particles made of synthetic or natural polymers, known as nanoparticles, are spherical in shape. Their sizes vary from 10 to 500 nm. These particles offer a wide range of possible uses due to their spherical form and high surface area to volume ratio. Nanoparticle size and surface characteristics have been studied to improve bioavailability, reduce clearance, and boost stability. By regulating these properties, the medication can now reach bodily tissue that might not have previously been reachable. Nanoparticles are divided into several categories based on their size, shape, and material qualities (Haleem et al., 2023).

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Furthermore, nanoparticles can be hard (such as titania [titanium dioxide], silica [silica dioxide] particles, and fullerenes) or soft (such as liposomes, vesicles, and nanodroplets). The classification of nanoparticles often relies on their use, such as in diagnosis or therapy vs fundamental research, or it may be connected to how they were generated. They have also aided in the development of new techniques of administering treatment, such as giving local warmth (hyperthermia), limiting vasculature to sick tissues and tumors, and transporting medication payloads (Al-Abduljabbar & Farooq 2023).

Magnetic nanoparticles have been used to trace the progression of cancer along lymph nodes in place of radioactive technetium. The nanoparticles function by taking advantage of the contrast change caused by microscopic particles of superparamagnetic iron oxide in magnetic resonance imaging (MRI). Such particles can also be utilized

to eliminate tumors by hyperthermia, which involves heating and destroying tissue on a small scale using an alternating magnetic field (Crintea et al., 2022).

Nanoparticles can be created to improve fluorescence imaging, positron emission tomography (PET), or ultrasound pictures. These strategies often need the nanoparticle's ability to recognize a certain cell or disease condition. The medication might be delivered by a nano capsule or a liposome, or it could be delivered in a porous nano sponge structure and then kept in place by bonding at the targeted spot, allowing for delayed drug release. The creation of nanoparticles to help with medicine delivery to the brain by inhalation offers great potential for the treatment of neurological illnesses such as Parkinson's, Alzheimer's, and multiple sclerosis (Jain et al, 2018). Nano formulation of drugs is one strategy to deliver pharmaceutical agents more precisely to the targeted tissue and reduce the overall dose and potentially toxic side effects (Choi et al., 2023).

Types of nano formulations: Nanocrystal: Nanocrystals have been utilized to deliver insoluble medicines like paclitaxel. PEGylation is a critical idea that extends the circulation duration of the nanocarrier system and enhances medication therapeutic outcomes (Sun et al., 2008).

Nanocapsule: This has the potential to increase medication stability and bioavailability. Peptides, hormones, proteins, enzymes, medicines, metabolites, or reporter molecules may be protected from biological and chemical degradation using nano capsules (Janeth et al., 2017).

Nanospheres: Nanospheres are used in anti-wrinkle creams, moisturizing creams, and anti-acne nanoparticle creams. Nanospheres are utilized to transport active ingredients deeper into the skin, as well as to preserve the active component from enzymatic or chemical destruction or to provide a regulated release. In the case of scents, this delivery mechanism was found to extend active release (Prieto et al., 2017).

Nanosponges: They can solubilize weakly water-soluble medicines, resulting in extended release and improved medication bioavailability. The two primary therapeutic applications for nanosponges are targeted drug delivery (ensuring that the medicine reaches the target cells in the body, such as cancer cells) and enhanced drug delivery, which allows for improved physical qualities of pharmaceuticals (e.g., solubility).

Nanoprecipitation: This technique involves quickly injecting a drug solution into an aqueous phase after it has been dissolved in a water-miscible organic solvent. Drugs precipitate quickly in aqueous media, forming nanoscale drug particles.

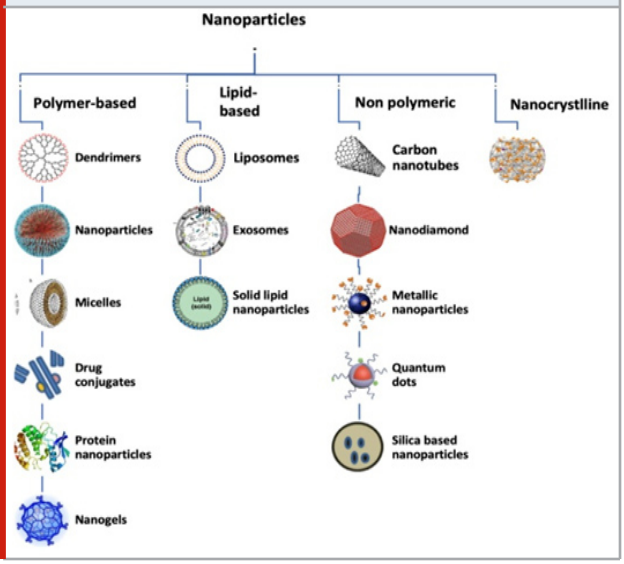
Nano Formulation Based on Emulsions: Preparing an oil-in-water or water-in-oil emulsion and then letting the solvent evaporate to produce nanoparticles is known as solvent evaporation. [16] It includes phase inversion and spray drying (Sun et al., 2022).

Coacervation: In this method, a polymer solution is phase separated into a coacervate phase, which contains the medication. Upon solidification of the coacervate phase, nanoparticles may develop.

Electronspinning: Electrospinning is mainly used to manufacture nanofibers, but with the right formulations, it can also be utilized to produce nanosized particles.

Technology of Supercritical Fluids: Supercritical Antisolvent Process: To precipitate nanoparticles from a solution, antisolvents such as carbon dioxide are employed at supercritical temperatures. [20] Supercritical Fluid Extraction of Emulsions: In order, to extract nanoparticles from an emulsion, supercritical fluids must first be generated.

Figure 1: Types of Nanoparticles.



Nano carriers used in nanoformulation: Materials known as nano carriers are made with the purpose of encapsulating and delivering medicinal medicines, imaging agents, or other payloads in a precise and regulated way. These carriers play a crucial role in nanoformulations, improving medication stability, bioavailability, and solubility while frequently enabling tailored administration (Marianna Foldvari 2010). The following are a few typical nano

Carriers found in nanoformulations:

Liposomes: Lipid bilayers form the spherical vesicles known as liposomes. In their lipid bilayers or aqueous core, they can contain hydrophilic or hydrophobic medications, respectively. Liposomes can be used for a variety of medication delivery applications because they are biocompatible and adaptable (Zhang et al., 2018).

Polymeric nanoparticles: Biocompatible and biodegradable polymers are used to create polymeric nanoparticles. They can be made to release medications gradually or under strict supervision. Chitosan nanoparticles and poly (lactic-co-glycolic acid) (PLGA) nanoparticles are two examples.

Micelles: Made up of amphiphilic molecules, micelles are self-assembling structures. When these molecules are present in concentrations higher than their critical micelle concentration (CMC), they form. Drug distribution can be improved when hydrophobic medications are dissolved in the center of micelles.

Nanocapsules: Having a core-shell structure, nanocapsules are nanoscale capsules. Drugs can be accommodated in the core, while proteins, polymers, or lipids are frequently found in the shell. It is possible to encapsulate both hydrophobic and hydrophilic molecules using this architecture.

Dendrimers: Having a distinct structure, dendrimers are highly branching macromolecules. Their size and surface functionality can be precisely controlled during their synthetic process. Drugs or imaging agents are frequently encapsulated inside of dendrimers.

Solid Lipid Nanoparticles: Solid Lipid Nanoparticles (SLNs) are room-temperature, lipid-based nanoparticles in a solid state. In comparison to conventional liposomes, they provide better stability and regulated release. Drugs can be shielded from deterioration by the lipid matrix.

Carbon nanotubes: Therapeutic compounds can be carried via carbon nanotubes, which are cylindrical structures with special features. Functionalized carbon nanotubes can be used as delivery systems for different payloads, such as imaging agents or drugs (Ganesh et al., 2015).

Metal nanoparticles: As carriers, metal nanoparticles derived from gold, silver, or iron oxide can be employed. Their surfaces can be functionalized for drug loading or targeting, and they may possess special features.

Cyclodextrins: Cyclodextrins are cyclic oligosaccharides that have ability to combine with hydrophobic medications to form inclusion complexes that increase the solubility of the former. They can serve as drug delivery vehicles, particularly for medications that are not very soluble in water (Patel et al., 2020).

Protein-based Nanoparticles: Drug delivery nanoparticles can be formed from proteins, such as albumin or gelatin. These protein-based carriers can be engineered to have particularly targeting characteristics and are biocompatible.

Applications of nano formulation: Applications for nanoformulations can be found in many different domains, and they provide benefits like focused therapy, increased therapeutic efficacy, and better drug distribution. It includes1) Drug Delivery 2) Targeted Drug Delivery 3) Sustained Release 4) Cancer Therapy 5) Imaging and Diagnostics 6) Vaccines 7) Gene Delivery 8) Cosmetics & Personal Care 9) Agriculture 10) Food and Nutraceuticals 11) Wound Healing 12) Environmental Remediation To guarantee safety, scalability, and regulatory compliance in these applications, however, further research is necessary.

Methods used to improve drug delivery in nano formulation: One of the most important facets of pharmaceutical research and development is enhancing medication delivery. A range of techniques and tools are used to improve medication delivery's effectiveness, safety, and specificity. Here are some essential techniques for

Enhancing medication delivery:

Nanotechnology: Using nanoscale carriers to encapsulate medications, such as liposomes, micelles, polymeric nanoparticles, and dendrimers. This improves stability and solubility and enables tailored distribution.

Systems of Lipid-Based Delivery: Lipid vesicles known as liposomes are capable of encasing medications that are hydrophilic or hydrophobic. They enhance the stability and solubility of drugs and can help with targeted distribution. Lipid-based nanoparticles with regulated drug release and improved bioavailability are called solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs).

Delivery Systems for Polymers:

Polymeric Nanoparticles: Nanoparticles for controlled medication release can be made from biodegradable and biocompatible polymers and targeted delivery.

Polymeric Micelles: Amphiphilic block copolymers self-assemble to generate self-assembling structures that improve the solubility of hydrophobic medicines.

Drug Pairs: Prodrug Design: The process of chemically modifying pharmaceuticals to produce prodrugs that, in their original form, are inactive or less active. In vivo activation enhances medication delivery and stability. Drugs and antibodies are linked to create antibody-drug conjugates (ADCs), which are then specifically delivered to target cells, such cancer cells. Encasing medication particles in microspheres or microcapsules to prevent deterioration and enable regulated release is known as microencapsulation.

Targeted Administration of Medicines: Active targeting is the process of delivering drugs to the intended location on cells by utilizing ligands, such as peptides or antibodies, to target particular receptors. Using the enhanced permeability and retention (EPR) effect in tumors, where leaky blood arteries allow nanoparticles to enter the body, is known as passive targeting. Using the increased permeability and retention (EPR) effect in tumors—where leaky blood arteries enable nanoparticles to aggregate preferentially in malignant tissues—is known as passive targeting.

Microneedle Technology: Transdermal drug delivery by microneedles allows for regulated release of medication by avoiding the epidermal barrier.

Electrospinning: This technique produces nanofibers that are used in tissue engineering, medication delivery, and wound healing.

Ultrasound-mediated delivery: The process of using ultrasound to improve drug penetration into tissues or cells, is referred to as ultrasound-mediated delivery.

Microfluidics: Using microfluidic devices to carefully manage the formulation process to produce nanoparticles or microcapsules with desired qualities is known as microfluidics.

Magnetic Drug Delivery: To improve targeted drug delivery, magnetic nanoparticles are guided to precise places using magnetic fields. Creating responsive systems that release medications in response to particularly stimulus, such as pH, temperature, or enzyme activity, is known as "smart drug delivery."

Routes of administration for nanoformulation: The drug's properties, the intended site of action, and the intended therapeutic outcome all influence the delivery method selection. The following are some typical medication delivery pathways for nanoformulations: Oral Administration, Intravenous Administration, Transdermal Delivery Intramuscular and Subcutaneous Injection Inhalation, Intrathecal and Intraventricular Administration, Intraperitoneal Administration, Ocular Delivery, Nasal Delivery, Delivery via Vagina and Rectal, Intradermal Delivery& Intraperitoneal Delivery. The advantages and disadvantages of each administration route are taken into consideration, while designing nanoformulations in order, to maximize drug delivery for certain therapeutic uses.

Factors that improve drug delivery in nano formulation: By using nanoscale carriers to address issues with drug solubility, stability, and targeted distribution, nanoformulations aim to improve drug delivery. The following elements influence how well drugs are delivered in nanoformulations:

Greater Surface Area: When compared to traditional formulations, nanoformulations offer a noticeably larger surface area. Better interactions with biological systems are made possible by the increased surface area, which enhances medication distribution and absorption.

Better Solubility: Hydrophobic medications' poor solubility is addressed via nanoformulations. Drug solubility and bioavailability are improved when drug particles are reduced to the nanoscale because this improves the effective surface area exposed to the surrounding medium.

Improved Bioavailability: Rapid drug absorption and distribution are made possible by the tiny particle size and larger surface area of nanoparticles, which enhances bioavailability. This is crucial for medications whose oral bioavailability is limited.

Long-term Sustained and Controlled Drug Release: Drugs can be released over an extended period of time with the use of nano formulations. This controlled release profile enhances patient compliance, lowers adverse effects, and maintains therapeutic medication levels.

Targeted Drug Delivery: Certain tissues or cells can get drugs in a targeted manner thanks to nano formulations. While passive targeting can be accomplished by the increased permeability and retention (EPR) effect in some pathological circumstances, such as tumor tissues, active targeting is facilitated by surface modifications using ligands or antibodies.

Protection of Drugs: Liposomes and nanoparticles are examples of nanocarriers that can shield pharmaceuticals from enzymatic or adverse environmental degradation. During transportation and storage, this protection improves the stability of medications.

Better Cellular Uptake: Drugs that have trouble crossing cell membranes can benefit from nano formulations, which can improve cellular uptake. It is possible to use a variety of methods, such as receptor-mediated endocytosis, to help drugs enter target cells.

Decreased Side Effects: In nano formulations, targeted medication administration and controlled release help to minimize off-target effects and lower systemic toxicity. This is especially helpful for cancer treatment and other illnesses where accurate medication localization is essential.

Biocompatibility: To guarantee that nano formulations are compatible with biological systems, biocompatible materials are frequently used in their creation. This lowers the possibility of negative reactions and raises the medication delivery system's safety rating.

Customized Surface Properties: By altering their surface, nanoparticles can be made to exhibit particularly characteristics like greater target cell contact, enhanced stability, or stealth behavior—a lower capacity to be recognized by the immune system.

Multifunctional Platforms: By combining therapeutic pharmaceuticals with imaging or diagnostic agents, nano formulations can function as multifunctional platforms. This allows for simultaneous diagnosis and therapy.

Administration Ease: Based on the demands of the patient and the properties of the medicine, nano formulations can be created for a variety of administration routes, such as oral, intravenous, transdermal, or inhalation. This flexibility in drug delivery allows for customized treatment plans. These variables must be carefully taken into consideration, keeping in mind the unique characteristics of the medication and the intended therapeutic objectives, so nano formulations to be applied successfully.

Nano formulation improving drug delivery: The following are some ways that drug distribution can be enhanced by nanoformulations:

Enhancement of Bioavailability: Poorly water-soluble medications can become more soluble thanks to nanoformulations, which increases their absorption and bioavailability. Better absorption of medications is made

possible by the protective action of nanoparticles against gastrointestinal tract degradation.

Targeted Administration of Medicines: Targeting particularly tissues, cells, or organs with functionalized nanoparticles can minimize off-target effects and enhance therapeutic results. Adding ligands to targets is known as active targeting. Attaching ligands to the nanoparticles that enable them to identify and bind to certain receptors on target cells is known as active targeting.

Prolonged Release: Drugs can be released from nanoformulations in a regulated or sustained manner, resulting in a longer duration of action and fewer dosage adjustments.

Defence of Pharmaceutical Molecules: Drugs can be more stable in biological settings by using nanoparticles to shield them from enzymatic or chemical processes that could break them down.

Delivery Within Cells: Drugs can be delivered intracellularly more easily with the help of nanoparticles, reaching their intended locations inside cells.

Diminished Adverse Reactions: By limiting the amount of time, medication is exposed to healthy tissues, targeted delivery can lower the likelihood of adverse consequences.

Combination Counselling: Co-delivery of several medications is made possible by nanoformulations, which enables combination therapy with beneficial effects.

Diagnostic Imaging: Nanoparticles can be employed as diagnostic instruments for illnesses or as imaging agents to see how drugs are distributed throughout the body.

Personalized Health Care: Personalized medicine can be advanced by customizing nanoformulations to each patient's unique set of traits.

Non-intrusive Administration Routes: As an alternative to more conventional delivery methods like oral or intravenous injection, nanoparticles can be engineered for non-invasive routes like transdermal or nasal distribution.

Cells/Tissues helping in drug delivery: Different cells and tissues can be used or targeted in medication delivery to improve the safety, effectiveness, and selectivity of medicinal medicines. The following tissues and cells are

Frequently used in medication delivery: The inner surface of blood arteries is lined with endothelial cells. It is possible to create nanoparticles to get through the endothelium barrier and deliver them specifically to particularly tissues or organs.

Macrophages: As a component of the immune system, macrophages can be used to carry drugs, particularly to inflammatory regions. It is possible to engineer nanoparticles such that they are specifically delivered to areas of infection or inflammation and are absorbed by macrophages.

Hepatocytes, or liver cells: Because the liver is involved in drug processing, it is frequently the target of drug delivery methods. It is possible to engineer nanoparticles so that they gather in hepatocytes, which would improve the administration of medications that the liver must metabolize or be used to treat liver illnesses.

Cancer Cells: One of the main goals of medication distribution is to target cancer cells. In order to minimize side effects, nanoparticles can be functionalized to recognize and deliver medications to cancer cells only, sparing healthy cells.

Immune cells: Vaccines and immunotherapies can be developed specifically targeting immune cells, such as dendritic cells. Antigens or therapeutic substances that elicit an immune response can be delivered using nanoparticles.

Central Nervous System (CNS) Cells: The blood-brain barrier makes it difficult to transfer drugs to the brain. It is possible to create nanoparticles that can get through this barrier, making the treatment of neurological conditions easier.

Skin Cells: Transdermal drug administration delivers medications locally or systemically by targeting the skin. Drugs can be progressively released from nanoparticles by making them able to permeate the layers of skin.

Bone Cells: Osteoporosis and bone cancer can be treated by targeted medicine delivery using nanoparticles to the bone tissue.

Mucosal Cells: Local medication administration or systemic absorption can be directed towards mucosal surfaces, such as those found in the respiratory and gastrointestinal systems. Through mucosal barriers, medication absorption can be improved by nanoparticles.

Tumor Vasculature: Drug delivery strategies can target the distinct features of blood arteries found within tumors. It is possible to engineer nanoparticles so that they selectively collect in tumor blood arteries, enhancing medication delivery to the tumor.

Red Blood Cells: To improve distribution to particularly organs and extend circulation periods, drug-loaded nanoparticles can be encapsulated or adhered to red blood cells.

Lymphatic System: Drug delivery to lymph nodes and tissues connected to the immune response is made possible by the ability of nanoparticles to specifically target the lymphatic system.

Synovial Cells: To administer anti-inflammatory medications to synovial cells in the joints, such as in rheumatoid arthritis, nanoparticles can be specifically targeted to these cells. Through the utilization of distinct cell and tissue properties, scientists can create drug delivery systems that optimize therapeutic effects while reducing

side effects. In order, enhance patient care, the discipline of nanomedicine is still investigating novel strategies for targeted drug delivery.

CONCLUSION

This review makes it clear that the use of nontechnology in medicine and drug delivery has created new avenues for individualized and secure treatment options. In the end, researchers are able to administer medications for longer periods of time with less frequent doses (sustained release), higher precision, and penetration in difficult-to-access tissues through the alteration of molecular size and surface features. There are many benefits to using micro and nanoparticles in biomedicine, particularly when it comes to drug delivery, over traditional methods which include improved drug delivery, high-performance properties of the product, using less costly drug concentrations in the delivery systems, extending the drug's bioactivity by shielding it from environmental effects in biological media, and more effective treatment with fewer side effects.

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Enlisting of Some Molluscan Species from Pujaritola Dam of Gondia Maharashtra, India.

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ABSTRACT

Freshwater molluscs are of the important components of aquatic ecosystem which help in maintaining the balance and cleanliness of aquatic environments. Some gastropods serve as intermediate host to transfer helminthic infections. This survey was carried out to study diversity of freshwater malacofauna of Gondia region from Maharshtra India. Survey was done by visual searching across the banks of Pujaritola dam. Molluscan shells were collected by hand picking method. Species identification was carried out using standard references. Total 12 species of molluscs have been reported in this paper, 9 of these are gastropods and 3 are bivalves. These species belong to 3 orders, 7 families and 7 genera. Gastropod species recorded were P*ila theobaldi, Pila pesmei, Gabbia orcula producta, Thiara scabra, Lymnaea acuminata form gracilior, Lymnaea acuminata* form *patula, Lymnaea biacuminata, Gyraulus convexiusculus,* and *Gyraulus rotula.* Bivalves found at site were *Corbicula striatella, Corbicula peninsularis* and *Sphaerium indicum. Lymnaea biacuminata* was rarest species at this site followed by *Pila theobaldi, Pila pesmei, Lymnaea acuminata* form *gracilior*. This study will contribute to carry out further investigations on the freshwater malacofauna's occurrence in this area as well as its analysis of distribution and richness. Three new species that had not before been documented in Maharashtra have been found during this survey. Understanding the region's molluscan species—both edible and harmful—will be aided by this inventory. This work will form the basis of further research in this area. There is further scope of research in implications in other field such as, food, disease transmissions and medicine.

KEY WORDS: PFRESHWATER MALACOFAUNA, GASTROPODS, BIVALVES, PUJARITOLA DAM, GONDIA DISTRICT.

INTRODUCTION

Phylum Mollusca is the second largest phylum after Arthropoda. Most molluscs are marine, with a small number of freshwater and some terrestrial species. After migrating from the ocean to freshwater, molluscs eventually arrived in terrestrial environments (Dey, 2007). Except Antarctica, freshwater molluscs are found all over the world. Freshwater molluscs are divided into class Gastropoda and class Bivalvia. In gastropods soft body is enclosed in a single, spirally coiled shell. In bivalves soft body is enclosed in two parts of shells which are hinged together at dorsal side by ligament. Morphological characters of shells in molluscs shows different characters that help in their taxonomical identification.

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Freshwater molluscs are very essential in aquatic ecosystem and have significant impact on biogeochemical cycle (Tripathy and Mukhopadhayay, 2015). Molluscs play important role in food chain, and they keep water clean by filter feeding mechanism (Dey 2008). Molluscs have great economic importance in food, freshwater pearl culture production, as medicines and various shell uses. Some freshwater molluscs acts as intermediate hosts for many trematode worms causing diseases in man, cattle, sheep, monkey, goat (Gadgil and Shah 1955; Mukherjee and Chauhan 1965). Various investigators do important work in documenting malacofauna throughout India. The Zoological Survey of India contributed great in this area.

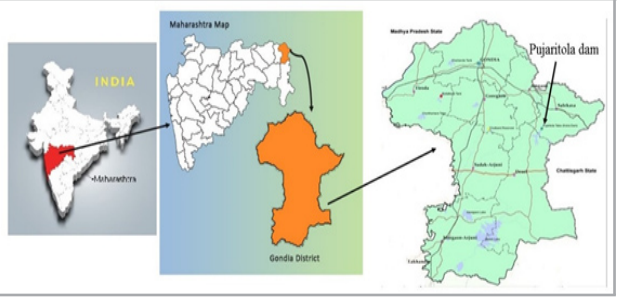
Diversity surveys have been done over the recent years in various regions of India by Pasupuleti and Subba Rao (2014), Sajan et al (2014), Kumar et al (2019), Rekha et al., (2021), Chutia and Kardong, (2021), Jadhav et al., (2023). Patil (2005) which have been carried out on freshwater molluscs in Melghat Tiger Reserve. In Nagpur region, Dorlikar, et al. (2014) and Wagh et al., (2019) have carried out investigations in Amravati region, where they

have worked on freshwater molluscan diversity. There is no previously conducted research on molluscan diversity in Gondia, hence the current study deals with the description of molluscans from Pujaritola dam of Gondia Maharashtra India.

MATERIAL AND METHODS

Study Area: Gondia district is in the north-eastern part of Maharashtra. Madhya Pradesh surrounds Gondia to the north and Chhattisgarh to the east. It lies at latitudes 20.39 and 21.380 North and longitudes 79.27 to 80.420 east. The district occupies an area of 5,234 km2. Gondia has extremely high summer temperatures and extremely low winter temperatures. During each rainy season the average recorded rainfall is over 1,200 mm. Its average relative humidity is 62 percent. The highest recorded temperature in May is 480 C and lowest in January 00 C.

Figure 1: The Map of Gondia Distric Showing Pujaritola Dam



Pujaritola dam also called as Kotra dam, is built across the Bagh River having earth fill construction, with main objective of irrigation. It is near Amgaon at 21.212119840 N latitude and 80.42404180 E longitude. Height of dam is 19.2 meter and length is 2,661 meters.

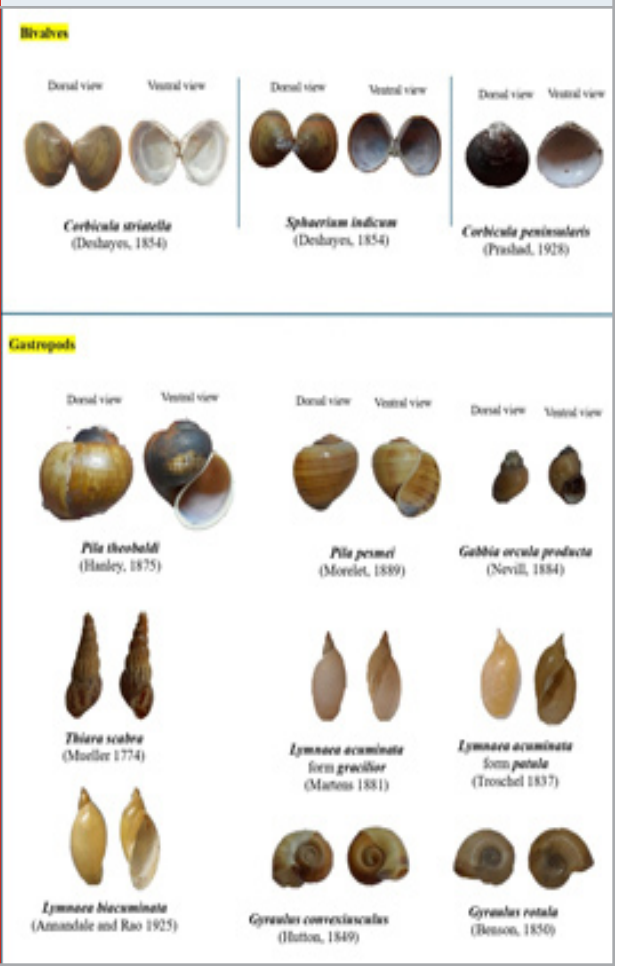
Field Sampling: Field sampling was done for six months from September-2023 to February-2024. Survey was done by visual searching across the banks of Pujaritola dam. Molluscan shells are collected by hand picking method from littoral zone of water body. Shells lodged in aquatic plants and weeds were collected by scoop net, forceps. Ony molluscan shells were collected for analysis. Live molluscs were observed only at site and were promptly released back in water bodies.

Collected molluscan shells were safely brought in polythene bags and plastic boxes to avoid breakage. They were washed properly with soft brush under tap water to remove dirt and were dried. Species identification was done by using standard keys of Subba Rao (1989), Ramkrishna and Dey (2007), and some of the recent research papers. Quantification of malacofauna was done by random quadrant method (Christian and Harris 2005). Quadrat used was 0.5 m length having 25 small square each square of 10 cm.

RESULTS AND DISCUSSION

Total 12 species of mollusks were reported in this paper 9 of these are gastropods and three are bivalves. These species belong to 3 orders , 7 families and 7 genera as shown in (Table-1). Gastropod species recorded were *Pila theobaldi, Pila pesmei, Gabbia orcula producta, Thiara scabra, Lymnaea acuminata* form *gracilior, Lymnaea acuminata* form *patula, Lymnaea biacuminata, Gyraulus convexiusculus,* and *Gyraulus rotula. Corbicula striatella, Corbicula peninsularis, Sphaerium indicum* were bivalve species recorded from site (Figure 2).

Figure 2: Colleted and Identified Moulluscs from Pujaritola dam Gondia District ,Maharashtra ,India.



Among these Sphaerium indicum , *Corbicula striatella* and *Corbicula peninsularis* were very commonly found species. *Gabbia orcula producta, Lymnaea acuminata* form *patula, Gyraulus convexiusculus, Gyraulus rotula* and *Sphaerium indicum* were common species at the site. *Pila theobaldi, Pila pesmei, Lymnaea acuminata* form *gracilior* were rare whereas *Thiara scabra* and *Lymnaea biacuminata* were very rarely recorded from Pujaritola dam. *Pila theobaldi* is used as food (Tripathy and Mukhopadhayay, 2015). *Gyraulus convexiusculus* is pathogenic species carried helminthic infection in man and pig (Mukherjee and Chauhan 1965).

Table 1. Classification of collected malacofauna from Pujaritola dam Gondia, Maharashtra, India.

Sr. No.	Name of Species	Reference	Local Status
Class – Gastropoda			
Order- Mesogastropoda			
Family – Pilidae			
1.	*Pila theobaldi* (Hanley, 1875)	Dey, A (2007); Subba Rao, N. V. (1989)	R
2.	*Pila pesmei* (Morelet, 1889)	http://www.applesnail.net/	R
Family -Thiaridae			
3.	*Thiara scabra* (Mueller 1774)	Dey, A (2007); Subba Rao, N. V. (1989)	VR
Family – Bithyniidae			
4.	*Gabbia orcula producta* (Nevill, 1884)	Dey, A (2007); Subba Rao, N. V. (1989)	C
Order – Basommatophora			
Family – Lymnaeidae			
5.	*Lymnaea acuminata form gracilior* (Martens 1881)	Dey, A (2007); Subba Rao, N. V. (1989)	R
6.	*Lymnaea acuminata form patula* (Troschel 1837)	Dey, A (2007); Subba Rao, N. V. (1989)	C
7.	*Lymnaea biacuminata* (Annandale and Rao 1925)	Dey, A (2007); Subba Rao, N. V. (1989)	VR
Family – Planorbidae			
8.	*Gyraulus convexiusculus* (Hutton, 1849)	Dey, A (2007); Subba Rao, N. V. (1989)	C
9.	*Gyraulus rotula* (Benson, 1850)	Dey, A (2007); Subba Rao, N. V. (1989)	C
Class – Bivalvia			
Order – Veneroida			
Family – Corbiculidae			
10.	*Corbicula striatella* (Deshayes, 1854)	Dey, A (2007); Subba Rao, N. V. (1989)	VC
11.	*Corbicula peninsularis* (Prashad, 1928)	Dey, A (2007); Subba Rao, N. V. (1989)	VC
Family- Pisidiidae			
12.	*Sphaerium indicum* (Deshayes, 1854)	Dey, A (2007); Subba Rao, N. V. (1989)	C
VC- very common, C- common, R- rare, VR- very rare			

CONCLUSION

This study will contribute to the investigation of the freshwater malacofauna's occurrence in this area as well as its analysis of distribution and richness. Three new species that had not before been documented in Maharashtra are found during this survey. Understanding the region's molluscan species—both edible and harmful—will be aided by this inventory. This work will form the basis of further research in this area. There is further scope of research in implications in other field such as, food, disease transmissions and medicine.

Conflict of interest: Authors declare no conflict of interest

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Trends of Cutaneous Leishmaniasis in Western Ethiopia: A Retrospective Study.

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ABSTRACT

In Ethiopia, cutaneous leishmaniasis is primarily caused by Leishmania aethiopica and less often by Leishmania Tropica and Leishmania major. There is a major prevalence gap in study areas. Hence, this study assessed the trends of cutaneous leishmaniasis in the western part of Ethiopia. A three-year retrospective study (09 October 2018 to 31 January 2022) was conducted by extracting information from the national leishmaniasis register for patients visiting the Nekemte Specialized Hospital (NSH) treatment center, Nekemte, Western Ethiopia. A standard data abstraction checklist was used to review Leishmaniasis records. Data were extracted from the national leishmaniasis cases registration book by principal investigators and summarized using Microsoft Excel. All data were entered and analyzed using the Excel Microsoft office package. A total of 64 patients were treated for cutaneous leishmaniasis in the area during the study period. Most of the cases were among those aged 15-24 years (39.1%) while extreme age groups reported the least. About 35 (54.69%) of cutaneous leishmaniasis cases were from rural areas, and two-thirds (31, 65.96%) of patients were seeking medical treatment after 3-6 months of developing signs and symptoms. One-fourth (17, 26.56%) of CL cases were reported in January followed by August (10, 15.63), and there were no cases reported in June and October.The most affected age groups are those 15-24 years and those from rural communities. January is the month most cases are reported and late coming to treatment and needs awareness creation.

KEY WORDS: TRENDS, CUTANEOUS LEISHMANIASIS, WESTERN ETHIOPIA, NEKEMTE SPECIALIZED HOSPITAL.

INTRODUCTION

Leishmaniasis is a parasitic protozoan transmitted to humans by the bite of infected female sand flies. It causes three main forms of leishmaniasis: visceral (the most serious form of the disease), cutaneous (the most common form), and mucocutaneous. Cutaneous leishmaniasis (CL), the most common form, accounts for about 95% of cases globally (WHO 2015). CL is an emerging uncontrolled and neglected infection affecting millions yearly. Most CL patients are living in low- to middle-income countries where limited healthcare budgets and a large burden caused by other ailments such as malaria, tuberculosis, and HIV (human immunodeficiency virus are prominent, (Henry et al 2015).

CL is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability or stigma. It is estimated that between 600,000 to 1 million new cases occur worldwide annually (WHO 2022). It is endemic in the tropics and neotropics. Despite its increasing worldwide incidence, but because it is rarely fatal, CL has become one of the so-called neglected diseases, with little interest by financial donors, public-health authorities, and professionals to implement activities to research, prevent, or control the disease, (Reithinger et al 2007).

It presents in 67 countries in the old World (Europe, Africa, Middle East, Central Asia, and the Indian subcontinent). When susceptible populations become exposed, CL may result in noticeable epidemics, such as in Burkina Faso, Ghana and new pockets in Ethiopia. CL is rarely fatal but it can cause substantial suffering because of the related stigma and the disfiguring scars it leaves in a number of cases. Accurate disease burden is challenging since misdiagnosis is common and there are no standard reporting guidelines. There is limited information regarding the magnitude of the cases in low and middle-income countries, including Ethiopia. The lack of epidemiological burden and distribution makes it difficult to advocate for control

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activities and further research to inform public health policy (Sunyoto 2019).

The ten countries with the highest estimated CL case counts, Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica, and Peru, together account for 70 to 75% of global estimated CL incidence. Approximately, 0.7 to 1.2 million CL cases occur each year, globally, Alvar et al (2015). In Ethiopia, CL is primarily caused by *Leishmania aethiopica* and less often by *Leishmania tropica* and *Leishmania major*. *Leishmania aethiopica* causes both diffuse cutaneous leishmaniasis (DCL) and localized cutaneous leishmaniasis (LCL), which are found in the highlands of Ethiopia. Leishmania aethiopica occurs only in the Ethiopian and Kenyan highlands and its reservoir is the rock hyrax, while the vector is *P. larroussius*, (Ngure et al 2009, Jirata et al 2006 and Momen and Cupolillo 2009).

The study conducted in ALERT (All African Leprosy Rehabilitation and Training Center) Hospital, Addis Ababa, Ethiopia showed that based on geographical areas of the patients' flow Addis Ababa (41%, 96/234) and Oromia (30.3%, 71/234) had highest leishmaniasis distribution. Besides, it revealed that among 71 CL cases reported from Oromia region, 7(9.9%) of them were from Wollega, (Bekele et al 2014). However, there is no study conducted in four Wollega Zones and western part of the country which reveals the trends of CLs. Hence, this assessment used to fill the gaps in dearth of information.

MATERIALS AND METHODS

Study design and settings: A three-year retrospective study was conducted by extracting information from the national leishmaniasis register for patients visiting the Nekemte Specialized Hospital (NSH) treatment center between October 09, 2018, and January 31, 2022, Nekemte, Western Ethiopia. Based on the reviewed records, patients were coming to NSH treatment center from different Zones and districts of Western Ethiopia. The study was conducted at NSH CL treatment center, located in Nekemte town, Western Ethiopia. This center provides services for patients coming from 17 districts of East Wollega Zone and neighbouring Zones like Horo Guduru, West Wollega, West Shoa, Buno Bedele, Ilu-ababor, and Kellem Wollega in western part of the country.

Study population and selection criteria: We conducted a retrospective analysis of CL patients' medical records at NSH treatment center between October 09, 2018, and January 31, 2022. The extracted data include demographic data; diagnostic laboratory results and clinical information that were collected and assessed from a total of 64 cutaneous leishmaniasis cases. Each data element was carefully collected to avoid redundancy.

Data collection method and tools: Demographic (sex, age, and residence) and clinical (duration of illness/sick before admissions, size of lesions, and treatment outcome) information, and laboratory results (parasitological and HIV testing) on all registered CL cases are systematically collected using standardized data abstraction forms. A standard data abstraction checklist was used to review NSH treatment center Leishmaniasis records from October 09, 2018, to January 31, 2022. Leishmaniasis register hospital records were reviewed by principal investigators.

Data analysis and interpretation: This retrospective analysis included the analysis of the reported CL cases using the available hospital records at the NSH CL treatment center, in western Ethiopia. Data were extracted from the national leishmaniasis cases registration book and summarized using Microsoft Excel. All data were entered and analysed using the Excel Microsoft office package. Categorical data were expressed using frequencies, percentages, and proportions with trend analysis when applicable. For continuous data, the median was used for expression.

Operational definition: Cutaneous Leishmaniasis: A protozoan infection and vector borne disease of human beings caused by Leishmania specious that affects the skin primarily (Birhanu et al 2019).

Trends of Cutaneous Leishmaniasis: The occurrence and distribution of the disease in the population at a given period (Birhanu et al 2019).

Ethical consideration: Ethical approval was obtained from East Wollega Zonal Health Department, Public and Health Emergency Management (PHEM) case team institutional review board. Consent was also sought from the hospital administration before being involved. Since the data were extracted form secondary data; formal consent (verbal or written) from parent/guardian was not required. Besides, we used patient identifiable codes to maintain the confidentiality of each patient.

RESULTS

Sociodemographic characteristics: Starting from October 09, 2018, to January 31, 2022, a total of 64 patients were treated for cutaneous Leishmaniasis (CL) at Nekemte Specialized Hospital treatment Centre. Of the total cases with CL, about 35(54.69%) were males and 29(45.31%) of them were females. The median age for sex was 18.5 years, while that of males and females were 19 years and 18 years, respectively. The maximum and minimum age for males was 3 years and 45 years, while that of females was 4 years and 50 years. The majority of the cases were from the Nekemte council (29, 45.31%), and East Wollega Zone (29, 45.31%).

Among 17 districts found in East Wollega Zone, CL cases were reported from 9 districts. More than half of CL cases (17, 58.62%) were reported from three districts of the Zone: -Gida Ayana (4, 6.25%), Leka Dulecha (5, 7.81%), and Jima Arjo (8, 12.5%). CL affected all age categories from 3 years to 50 years, with a median age of 18.5 years. Most of the cases were those aged 15-24 years (39.1%), followed by 5-14 years (26.6%), while extreme age groups reported the least.

Table 1. Demographic and clinical characteristics of patients with cutaneous leishmaniasis (CL) based on Nekemte Specialized Hospital CL treatment center record review, Western Ethiopia, 2022(n=64).

Variables	Category	Frequency(n)	Percentage (%)
Sex	Male	35	54.69
	Female	29	45.31
	Total cases	64	100
Age group(years)	<5	3	4.7
	5-14	17	26.6
	15-24	25	39.1
	25-34	11	17.2
	35-44	3	4.7
	45-59	5	7.8
	Total cases	64	100
Residences	Urban	29	45.31
	Rural	35	54.69
	Total	100	100
	Nekemte council	29	45.31
	East Wollega Zone	29	45.31
	Others*	6	9.38
	Total cases	64	100
	< 3 months	15	31.91
Number of months sick before admission(**n=47 cases**)	3-6 months	31	65.96
	>6 months	1	2.13
	Total cases	47	100
Size of lesions(**n=14 cases**)	< 4 cm	11	78.57
	≥ 4 cm	3	21.43
	Total	14	100

Table 2: Age and sex distribution of patients with Cutaneous Leishmaniasis at Nekemte Specialized Hospital treatment center from October 09, 2018 to January 31, 2022, Nekemte, Western Ethiopia, 2022(n=64).

Age group(years)	Sex category					
	Male	%	Female	%	Total	Percentage (%)
<5	2	66.67	1	33.33	3	4.69
5-14	8	50	8	50	16	25
15-24	13	50	13	50	26	40.63
25-34	9	81.82	2	18.18	11	17.19
35-44	1	33.33	2	66.67	3	4.69
45-59	2	40	3	60	5	7.81
Total	**35**	**54.69**	**29**	**45.31**	**64**	**100**

Figure 1: Distribution of CL cases by districts in East Wollega Zone, Nekemte, Western Ethiopia, 2022(n=29).

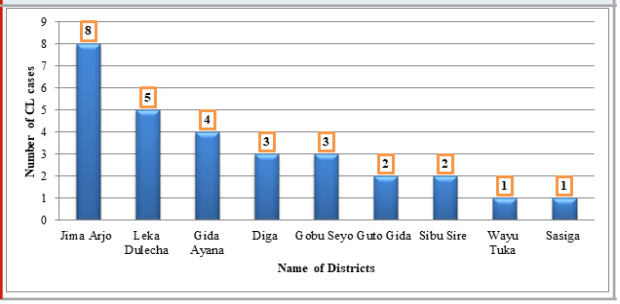
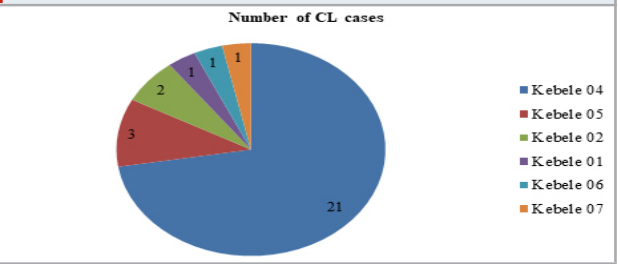


Figure 2: Distribution of CL cases by Kebeles in Nekemte council, Nekemte, Western Ethiopia, 2022(n=29).



More than half of CL cases (35, 54.69%) were from rural areas. About two-thirds (31, 65.96%) of patients were sick of medical treatment between 3-6 months, and in more than three-fourths (11, 78.57%) the size of the lesion was less than four centimetres [Table 1].

Figure 3: Line graph showing the distribution of cutaneous leishmaniasis cases by several months sick before admission based on record review, Nekemte, Western Ethiopia(n=47),2022.

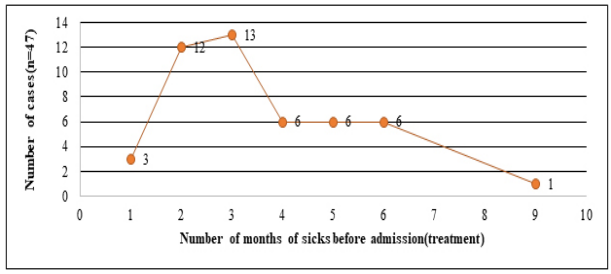
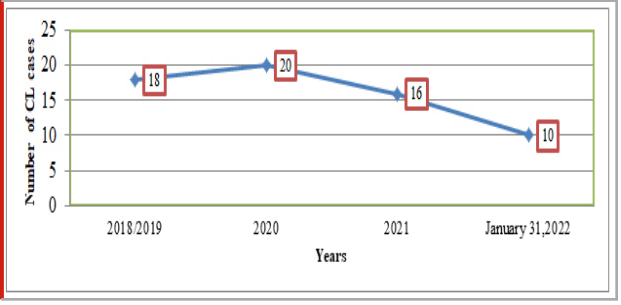


Figure 4: Line graph showing the trends of cutaneous leishmaniasis cases based on Nekemte Specialized Hospital treatment center record review, Nekemte, Western Ethiopia, 2022.



Age and Sex distribution of CL cases: This assessment indicated that young children and adolescents (15-34 years) of school-going age were disproportionately affected (37, 57.81%). The sex structure of patients with cutaneous leishmaniasis shows an almost one-to-one ratio (1.21: 0.81) [Table 2].

Table 3: A Quarter trends of reported CL cases based on Nekemte Specialized Hospital treatment center record review, Western Ethiopia, 2022.

Quarter and lists of months under it		Number of CL cases(n=64)					
		2019	2020	2021	2022	Total	
						Frequency(N)	Percentage
Quarter one	July	3	0	3	NA	6	31.58
	August	3	1	6	NA	10	52.63
	September	3	0	0	NA	3	15.79
	Sub-total	**9**	**1**	**9**	NA	**19**	**29.69**
Quarter Two	October	0	0	0	NA	0	0
	November	0	5	0	NA	5	50
	December	3	0	2	NA	5	50
	Sub-total	**3**	**5**	**2**	NA	**10**	**15.63**
Quarter Three	January	6	1	2	8	17	51.52
	February	6	2	0	0	8	24.24
	March	5	3	0	NA	8	24.24
	Sub-total	**17**	**6**	**2**	**8**	**33**	**51.56**
Quarter Four	April	0	1	0	NA	1	50
	May	0	1	0	NA	1	50
	June	0	0	0	NA	0	0
	Sub-total	**0**	**2**	**0**	NA	**2**	**3.13**
Grand-total		**29**	**14**	**13**	**8**	**64**	**100**

Distribution of CL cases among East Wollega Zone Districts: Among the total 64 CL cases reviewed from the hospital record, about (29, 45.31%) of CL cases were from East Wollega Zone districts. Among 17 districts found in East Wollega Zone, CL cases were reported from 9 of them. More than half of CL cases (17, 58.62%) were reported from three districts of the Zone: -Gida Ayana (4, 6.25%), Leka Dulecha (5, 17.24%), and Jima Arjo (8, 27.59%) [Figure 1].

Distribution of cases by kebeles of Nekemte council: The study showed that nearly three-fourths (21, 72.41%) of cutaneous leishmaniasis cases were reported from Kebele 04 of Nekemte council. Besides, the least was reported from Kebele 01, 06, and 07[Figure 2].

Number of months' sick before admission: The median number of months sick before admission was 3 months, while the minimum was 1 month and the maximum was 9 months. Nearly more than one-quarter of patients seeks care within 2 months (12, 25.53%) and 3 months (13, 27.66%) respectively. But, a significant number of patients (13, 27.66%) seek medical care greater than or equal to 5 months at Nekemte Specialized Hospital treatment center, Ethiopia. From those late medical treatment seekers, most of them (7/13, 53.85%) were from Nekemte town [Figure 3].

Yearly trends of Cutaneous Leishmaniasis in Western Ethiopia: This assessment showed that the number of cutaneous leishmaniasis was slightly increased from 2018/2019 to 2020 and then declined from 2020 to 2021in the last more than 2 years. The total number of CL cases was 18, 20, 16, and 10 from October 09, 2018, to January 31, 2022, respectively [Figure 4].

Quarterly trends of Cutaneous Leishmaniasis in Western Ethiopia: The study revealed that half (33, 51.56%) of CL cases were reported in quarter three (January, February, and March), followed by quarter one (July, August, and September) which was about (19, 29.69%) CL cases reported [Table 3].

Cumulative monthly trends of Cutaneous Leishmaniasis in Western Ethiopia: The study revealed that one-fourth (17, 26.56%) of CL cases were reported in January followed by August (10, 15.63). Besides, there were no cases reported in June and October [Figure 5].

Monthly trends of Cutaneous Leishmaniasis in Western Ethiopia: The study revealed that there was a case build up in the months of July (6 cases), February (8 cases), March (8 cases), August (10 cases), and January (17 cases) [Figure 6].

Note: - The date for the year 2022 was only up to January 31, 2022 and the rest of the months in the year filled zero (0) when sketching the line graph.
The study also indicated that all the patients were cured and there were no admitted cases. Besides, it showed that HIV testing was offered and performed for all the patients and there was no positive case found.

Figure 5: Line graph showing the cumulative monthly trends of cutaneous leishmaniasis cases based on Nekemte Specialized Hospital treatment center record review from 2019 to January 31, 2022, Nekemte, Western Ethiopia, 2022.

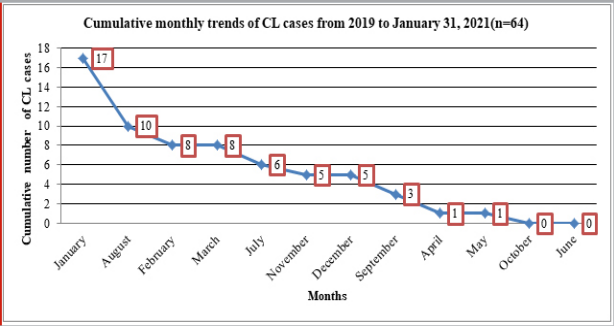
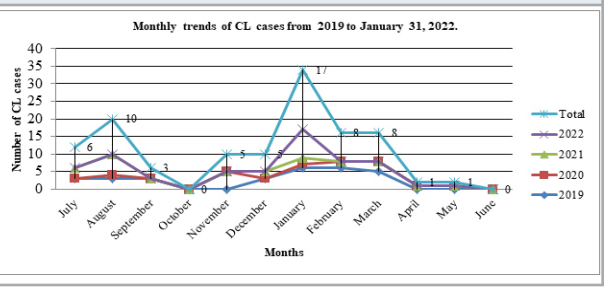


Figure 6: Line graph showing the monthly trends of CL cases reported based on Nekemte Specialized Hospital treatment center record review from 2019 to January 31, 2022, Western Ethiopia.



DISCUSSION

This study revealed that the trends of CL cases in western Ethiopia were fluctuating; it was slightly increased from 2018/2019 to 2020 and then declined from 2020 to 2021, which is in line with the ten-year trend analyses report in the University of Gondar Hospital, Northwest Ethiopia (Jejaw et al 2021). In contrast to this, the study conducted in Northeast Ethiopia revealed that the number of CL cases was increased significantly from year to year, (Birhanu et al 2019). These could be due to the differences in socio demographic and economic characteristics of the populations. Our study revealed that males were more affected by CL than females which agree with different studies, (Bekele et al 2014, Birhanu et al 2019, Jejaw et al 2021, Ngere et al 2016, Fikre et al, 2017, Eid, et al 2018, Amin, et al 2013, Bsrat et al 2015).

However, our finding is analogized with the studies reported from Algeria, Istanbul, Turkey, and Larstan, South of Iran that excess in the frequency of CL cases among females than males, (Benikhlef et al 2021, Sirekbasan et al 2021 Abolghazi et al 2019). In contrast to this, the studies conducted in rural communities in Tigray, northern Ethiopia, Silti woreda, Ethiopia, and Pakistan reported no gender difference in the frequency of CL cases, (Yohannes and Boelee 2019, Negera et al 2008, Kalyani et al 2021).

The possible explanation for this is due to the high engagement of males in outdoor activities like farming and different agricultural works compared to females. In this study, more than half of CL cases (35, 54.69%) were from rural areas which are incomparable with the study done in ALERT(All African Leprosy Rehabilitation and Training Center) Hospital, Addis Ababa, Ethiopia, and Larstan, South of Iran which showed that the distribution of CL patients was higher among rural than urban, (Bekele et al 2014, Birhanu et al 2019, Jejaw et al 2021, Ngere et al 2016, Fikre et al, 2017, Eid, et al 2018, Amin, et al 2013, Bsrat et al 2015).

In contrast to this, the study conducted in Northeast Ethiopia, and in Silti woreda, Ethiopia revealed a higher distribution of CL cases in urban areas than in rural ones, Birhanu et al 2019, Negera et al 2008). This variation could be due to the migration of infected CL cases from rural to urban and the higher prevalence of the cases in highland areas than in lowland areas of the study sites.This study also revealed that most of the CL cases were among those aged 15-24 years (39.1%), followed by 5-14 years (26.6%).

These indicated that young children and adolescents (15-34 years) of school-going age were disproportionately affected (37, 57.81%) by CL cases which is comparable with studies conducted in different areas, Bekele et al 2014, Amin, 2013 and Bsrat, 2015, Sirekbasan 2021, Yohannes and Boelee 2019, Negera et al 2008, Kalyani et al 2021). In this study, extreme age groups, less than 5years and greater than 45 years were the least affected by CL cases which is comparable with the study conducted at the University of Gondar Hospital, Northwest Ethiopia (Jejaw 2021).

In this study, about two-thirds (31, 65.96%) of patients were sick of medical treatment between 3-6 months. It is almost similar to the study reported in Northeast Ethiopia in which nearly half (49.1%) of the cases had persistent lesions for less than six months before seeking medical care and in the University of Gondar, Ethiopia in which the median (IQR) duration of lesion before visiting the hospital was 12 (6-24) months (Birhanu et al 2019 Fikre 2017).

However, this finding is shorter than the report of the study conducted in Kenya in which the median duration of illness was 2years (range 1-4 years), and in ALERT Hospital, Addis Ababa in which most of the CL patients were visiting the hospital after 12 months following the appearance of the lesion, (Negere 2020 and Bekele et al 2014).

This study revealed that there was a case build-up in July (6 cases), February (8 cases), March (8 cases), August (10 cases), and January (17 cases). About one-fourth (26.5%) of CL cases were reported in January, followed by August 10(15.63%). These are in line with the study conducted in Northeast Ethiopia, in which the cumulative monthly distribution of the disease was observed primarily in July (20.6%), June (14.2%), and January (11.7%); in Al Hassa, Saudi Arabia in which the number of cases showed a steep increase starting from November, reached a peak during January and February. The study conducted at the University of Gondar Hospital, Northwest Ethiopia, in which the highest prevalence rate (63.8%) was reported in September, followed by January (59.7%) and May (56.9%), (Birhanu et al 2019, Amin et al 2013, Jejaw et al 2021).

The study showed that there were no cases reported in June and October. In contrast to this, the study conducted in Pakistan showed that the highest peak observed was in June and September and in Kenya, which revealed that there was an occasional case peak in June, and at the University of Gondar Hospital, Northwest Ethiopia, which showed that the least percentage (49.3%) was reported in June, (Kalyani et al 2021, Jejaw et al 2021 Ngere 2020).

In this study, there was variation across the seasons in which more than half (51.56%) of CL cases were reported in winter (January and February), followed by summer (July and August) seasons (29.6%). In contrast to this, the study conducted in Larstan, South of Iran, showed that maximum infection reports of 1,315 (26.06%) were in autumn in 2010, and the minimum infection reports of 160 (3.23%) were in winter of 2013 (Abolghazi 2019). These could be explained by the fact that there might be differences in geographical characteristics and climatic conditions.

Limitations of the study: As a limitation, the study was retrospective in design. Second, hospital records available for the review were either incomplete or inaccurate. As it was secondary data, essential variables such as environmental conditions, socioeconomic status, demographic, and human behaviours were not collected. These could lead to underestimating or overestimating the burden of cutaneous leishmaniasis in the study area. In addition to this types of lesions and sites of CL infection were not recorded. Finally, types of CL including diffused, localized or mucocutaneous were not assessed. The result should, therefore, be interpreted with consideration of the limitation.

CONCLUSION

This study revealed that the trends of CL cases were fluctuating for the last more than three consecutive years (09 October 2018 to 31 January 2022) in the study area and continued as a public health problem in western Ethiopia. Those who live in rural areas, children and adolescents of school-going age were disproportionately affected. Hence, health care workers at different levels should give health education and/or health information to the rural and school communities in the study areas. This study revealed that there was a delayed seeking of medical treatment after being infected. Hence, health care professionals should give health education and promotion to improve the health-seeking behaviour of the communities. Besides, CL prevention and control interventions programs should be implemented by primary health care workers at grass root levels of the study areas in general and rural areas. Moreover, community-based research programs to determine the exact incidence and prevalence of CL cases and associated risk factors are needed.

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Ethics approval and consent to participate: Approval letter of permission was obtained from East Wollega Zonal Health Department, and the NSH medical director before the study was conducted to comply with the ethical guidelines. We used patient identifiable codes to maintain the confidentiality of each patient.

Consent for publication: 'Not applicable'

Availability of data and materials: The finding of this study was generated from the data collected and analyzed based on the stated methods and materials. The original data supporting this finding are available from the corresponding author upon reasonable request.

Competing interests: The authors declare that they have no competing interests

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