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ARTICLE

# Protective Roles of Some Leafy and Non-leafy Vegetables against the Severity of Arsenic-induced Skin Lesions among Women Living in Rural Bangladesh: A Case Control Study

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ABSTRACT

Arsenicosis is common among villagers as they drink more contaminated-water since the arsenic-crisis in Bangladesh. Supplementation of vitamins and micronutrients in counteracting arsenic toxicity has been proved for arsenic treatment. This study was intended to assess protective and beneficial roles of some commonly eaten vegetables on the development and severity of arsenic-induced skin lesions. A case-control study among (N=122) adult rural-women (62 cases had various forms of arsenical skin-lesions e.g. melanosis/keratosis/mixed-lesions and 60 sex-age-matched healthy-controls) was conducted in Shaharstee Upazilla of Chandpur district, Bangladesh. Socio-demographic data recorded in a pre-tested-questionnaire, 'per-day vegetables ingestion' of cases and controls were measured qualitative and quantitatively (24-hour recall-methods, food-frequency/week and food history-record/week). Multiple logistic regression/MLR analyses were performed to find out protective roles of some dietary leafy-vegetables/LVs and non-leafy vegetables/NLVs on arsenicosis and their influences on the degree of severity of arsenicosis also determined. Abstinence from taking some LVs/NLVs among cases than controls is associated with increased risk for arsenicosis ($P<0.05$). Amongst all most-frequently eaten vegetables (n=17) per day *Momordica diocia* has the highest skin protective role on arsenicosis [Adjusted odds ratio/AOR 8.2, 95% CI (2.11-31.9), P=<0.01], followed by *Ipomoea acquatica* (AOR:7.3), *Basella alba* (AOR:6.2), *Solanum tuberosum* (AOR:4.0), *Vigna unguiculata sesquipedalis* (AOR:3.2), *Trichosanthes anguina* (AOR:1.2) and *Abelmoschus esculentus* (AOR:1.2). Moreover, severe skin lesion was observed as compared to non-severe cases (mild/moderate) for less intake frequencies of vegetables. This study outlined that commonly eaten vegetables have protective and beneficial roles on arsenic-induced skin lesions. Large samples longitudinal study of this important field of therapeutic-intervention is warranted.

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## 1. Introduction

Arsenic is a common environmental toxin. Exposure to arsenic (particularly its inorganic form) through contaminated food and drinking water is an important public health burden worldwide and is associated with increased risk of skin keratosis, neurotoxicity, congenital anomalies, and cancer [1].

Bangladesh is the worst arsenic-affected country in the world and 'arsenic poisoning in drinking water' is her major public health problem especially among rural habitants since its first official disclosure in 1993 [2-5]. Since then, Bangladesh has been fighting an un-equal battle due to lagging of sustainable surveillance of arsenic-safe water supply and effective arsenicosis management for arsenic-exposed populations as eighty percent of peoples live in villages and their daily consumption of arsenic contaminated water is also high (> 5 liters per day) [5]. Study reported 12.6% of examined samples of water were arsenic contaminated in Bangladesh [6]. As arsenicosis case is diagnosed by only the presence of characteristic 'skin lesion', so in spite of having arsenic-contaminated tube-well water, sub-clinical arsenic-induced health problem is being underreported [5], although some improvements (increased awareness and less consumption of Arsenic-contaminated drinking water) cannot be denied [4]. World Health Organization (WHO) warned that few millions people of Bangladesh may suffer from arsenic-induced skin cancer over the next few decades if arsenic-free safe drinking water (<10 μg/L) is not ensured [7].

Skin lesion is one of the most common sign of chronic arsenic exposure with arsenic concentration of >100 μg/L, which can occur within month or after several years of exposure and causes melanosis (early and common manifestation like hyperpigmentation, spotted pigmentation, depigmentation, and hypopigmentation), skin cancer (basal cell and squamous cell carcinoma/ Bowen's disease) and keratosis (advanced stage of arsenicosis), which especially appears on palm and sole in different manner as a late feature of arsenical-dermatosis, and later on transformed to squamous cell carcinoma [8]. It has been found that the risk of skin lesion did not decrease after reducing the exposure for up to several years [9]. Among the medical measures, remedial role of some foods, supplementation of vitamins and micronutrient in counteracting arsenic toxicity has been proved [5]. Medicinal plants have been used since prehistoric times to treat different types of diseases, due to the presence of bioactive components [10]. Both leafy and non-leafy vegetables have intrinsic active nutrients (especially antioxidant vitamin A, E and C, B-complex (B1, B2, B5, B6, folic acid), linoleic acid, polysaccharides, calcium, manganese, magnesium, iron, and antioxidant minerals copper, zinc and selenium and abundant sources of bioactive plant's secondary metabolites like flavonoids and phenolic compounds (e.g. catechin, quercetin, Lectins, Pectins, and Glycosylated compounds) [11].

Both phenolics and flavonoids have antioxidant, immune-modulatory, anti-proliferative and pro-apoptotic, antibacterial, anticancer properties [12]. Quercetin, an important flavonoid has immense free radical scavenging ability toward superoxide anions, peroxyl, and hydroxyl radicals, thus, reducing oxidative stress and inflammation and accelerated wound closure in cell scratch assay, reduced pro-inflammatory cytokine production, and immune cell infiltration, and help to regenerate the mucosal layer after injury [13-15]. Study [16] reported dietary B-vitamins (particularly $B_1$, $B_6$, $B_{12}$) intake is associated with lower urinary mono-methyl arsenic and oxidative stress marker among US adults. Moreover, a recent trial [1] reported taking folic acid supplements, alone or in combination with other nutrients, might reduce blood arsenic and plasma homocysteine concentrations, potentially could facilitate arsenic methylation and excretion, thereby reducing arsenic toxicity in adults who had been exposed to arsenic-contaminated drinking water, compared to placebo. Previous study in Bangladesh reported arsenic induced skin lesions are more prevalent in men compared to women due to less efficient methylation of arsenic among men [17]. Study addressing roles of vegetable on arsenical skin-lesion is very scanty in Bangladesh and one decade earlier [18] and showed diversified diet rich in 'gourds and root' vegetables reduced arsenic-induced skin lesion. To the best of our knowledge no study explicitly considered the protective roles of only vegetables (both leafy-vegetables/LVs and non-leafy vegetables/NLVs) against arsenic-associated skin lesions among rural women. Thus, understanding the importance of availability, higher intake rate of vegetables in rural areas, also as a source of cheapest nutrients, potential bioactive and nutraceuticals properties [3,12,19-33], this study perceived to find out how protective roles of vegetables can play against the severity of arsenic-induced skin lesion. Findings of this study may assist understanding and addressing the remedial measure of higher-rate of arsenicosis among rural people and include proper steps at policy/or intervention-levels.

## 2. Methodology

### 2.1 Study Population and Site

'Source-population' of this 'case-control' study was all female population in nine villages out of three unions in Shaharastee Upazilla of Chandpur district, Bangladesh. Study place and population was selected according to the latest information obtained from the respective wing of the Directorate General of Health Services (DGHS), Bangladesh about the level of arsenic in drinking water. The report identified Shaharastee Upazilla as one of the worst affected area with arsenicosis [4].

## 2.2 Inclusion Criteria and Ethical Approval

Every cases were randomly selected on the basis of defined criteria which were willing to participate in the study, married women living in the study area for 2 years/ or more, sources of drinking water must from tube wells, which at least once had been marked "red" by the DPHE person and having visible signs of skin lesions (hypo pigmentation/or melanosis, scaling/or keratosis and mixed lesions) attributable to arsenic in drinking water after 'clinical assessment'. Exclusion criteria were subject having skin lesions but not arsenic-induced/or having other serious diseases, whose drinking water source could not be ascertained properly and not willing to take part in the study.

Written consent was obtained from all participants according to Helsinki declaration before the beginning of the study and confidentiality was marinated for all subjects. The study proposal was reviewed and approved by the Ethical Board of the Institute of Child and Mother Health (ICMH), Matuail, Dhaka, Bangladesh.

## 2.3 Sample Size Estimation and Collection of the Data

By using a standard formula [34] sample size per group (case/or control) was 58, and total sample size (both groups) was calculated 116≈122, considering expected proportion in controls=0.05, assumed odds ratio=5.5, confidence interval (CI) =0.95, and power=0.8. Thus 62 cases having arsenicosis and 60 sex-age-matched healthy controls without skin lesions (adult women ≥18 years) were selected from the same study area. Control (non-cases samples of the source-population) was also confirmed clinically for not having arsenicosis. Cases were categorized according to the severity (mild/moderate or severe) of skin lesions.

### 2.3.1 Socio-economic and Arsenic Related Data

A pretested (pretested on (n=10) subjects were discarded) structured questionnaire was used to record the data (age, education, occupation, drinking/cooking water sources, duration and severity of arsenicosis) by direct interview of the cases and controls with same questionnaire, in the same manner. Three of the authors personally interviewed all participants (both cases and controls). Data were collected under strict supervision and collected data in the questionnaire were checked by day to day basis.

### 2.3.2 Dietary-intake Information

The study was aimed to determine whether there is any protective role of different dietary vegetables (LVs/NLVs) with severity and the development of arsenic-induced skin lesions. Both qualitative and quantitative approaches were applied to measure habitual dietary practices in terms of taking vegetables. Food consumption [35] especially vegetables (both LV and NLVs) intake frequency per day at individual level was assessed for all 122 subjects (both case and control) by recording previous diet history, one-24 hour food-recall, and food frequency questionnaire (FFQ) for a week with the aid of portion size display. All food intake frequencies and food data were coded using food conversion tables [36] and then entered into computer for food analysis.

## 2.4 Statistical Analysis

Data (both socioeconomic and food intake) were analyzed by using Statistical Package (SPSS Inc, Chicago, IL, USA, version 23.0). Descriptive statistics were employed and values were expressed as frequency, percentage, mean and standard deviation as and where necessary. As total seventeen (n=17) vegetables were frequently taken, simple logistic regressions (SLRs) have been used to examine possible associations between exposure (Daily Ingestion of leafy (n=08)/ non-leafy vegetables (n=09): Intake/or no-intake) and outcome (having arsenic-induced skin lesions: Yes/No). Multiple logistic regressions (MLR) were performed adjusting demographic variables, odds ratios (ORs) and 95% confidence-interval (CI) were obtained to identify potential roles of leafy/non-leafy vegetables ingestion (by comparing case and control) associated with arsenic-induced skin lesions. Among seventeen (n=17) LVs and NLVs which were found statistically significant ($P<0.10$) in SLR were introduced in the MLR-analysis and the models were developed by 'Backward-Stepwise-Elimination**.** Hosmer-Lemeshaw goodness-of-fit and Nagelkarke-pseudo-$R^2$ of the models were also observed. Moreover, cross-classification-analysis ($\aleph^2$-test) have been used to examine possible associations of daily leafy/ or non-leafy vegetables intake in terms of severity of arsenic-induced skin lesions between severe and non-severe (mild/moderate) case-groups. P-value <0.05 was set as significance.

## 3. Results

### 3.1 Background Characteristics, Pattern, Duration and Severity of Arsenicosis

Mean ages (37.32±10.7 verses 35.73±9.7 years) and occupation (90% housewives) of both groups e.g. cases (village women having arsenicosis) and control (age-sex-matched healthy-controls without arsenicosis) are similar (P>0.05). Sources of drinking water for most of the cases (83.9%) were Hand-pressed tube well and for most of the control (91.7%) it was Pond/river/others sources. Consequently, who suffered from severe skin lesions (n=33), all (100%) used 'Hand-pressed tube-well' as drinking water sources, followed by 63.2% (n=12) and 70% (n=07) used 'Hand-pressed tube-well' respectively by mild-skin-lesion and moderate-skin-lesion sufferers. Majority (46.8%) of the cases had rain drop lesions or white cluster (of them, 60.6% severe, 40% moderate and 26.3% had mild skin lesion) followed by Hypopigmentation (27.4%) and Palmar keratosis (25.8%). Vast majority (83.9%) of cases had arsenicosis for <4 years (1.2±0.5) and mostly (82.3%) was diagnosed by doctor and NGO workers (6.1%) (Table 1).

**Table 1.** Background characteristics of all participants and pattern, duration and severity of arsenic induced skin lesions among cases

| Characteristics | Severity of arsenicosis of cases n (%) | | | Total cases | Controls (n= 60) |
|---|---|---|---|---|---|
| | Not severe (n=29) | | | | |
| | *Mild* (n=19) | *Moderate* (n=10) | Severe (n=33) | (n=62) n (%) | n (%) |
| **Age** | | | | | |
| ≤20 | 05 (26.3) | 00 (0.0) | 00 (0.0) | 05 (8.1) | 02 (3.4) |
| 21-30 | 04 (21.1) | 05 (50.0) | 05 (15.1) | 14 (22.6) | 20 (33.3) |
| 31-40 | 05 (26.3) | 03 (30.0) | 12 (36.4) | 20 (32.3) | 26 (43.3) |
| 41-50 | 05 (26.3) | 02 (20.0) | 10 (30.3) | 17 (27.4) | 06 (10.0) |
| ≥51 | 00 (0.0) | 00 (0.0) | 06 (18.2) | 06 (9.6) | 06 (10.0) |
| **Mean ±SD** | 37.32±10.7* | | | | 35.73±9.7* |
| **Occupation** | | | | | |
| House wife | 19 (100) | 09 (90.0) | 29 (87.9) | 57 (91.9)** | 55 (91.7)** |
| Service | 00 (00) | 01 (10.0) | 04 (12.1) | 05 (8.1) | 05 (8.3) |
| **Sources of drinking water** | | | | | |
| Hand-pressed tube-well | 12 (63.2) | 07 (70.0) | 33 (100.0) | 52 (83.9) | 11 (18.3) |
| Pond/river/others | 07 (36.8) | 03 (30.0) | 00 (0.0) | 10 (16.1) | 60 (91.7) |
| **Patterns of skin lesion** | | | | | |
| Rain drop lesions (white cluster) | 05 (26.3) | 04 (40.0) | 20 (60.6) | 29 (46.8) | NA |
| Hypopigmentation | 06 (31.6) | 05 (50.0) | 06 (18.2) | 17 (27.4) | |
| Palmar keratosis | 08 (42.1) | 01(10.0) | 07 (21.2) | 16 (25.8) | |
| **Duration (years) of arsenicosis** | | | | | |
| <4 | 14 (73.6) | 08 (80) | 30 (90.9) | 52 (83.9) | NA |
| ≥4 | 05 (26.4) | 02 (20) | 03 (9.1) | 06 (16.7) | |
| **Mean ±SD** | 1.2±0.5 | | | | |
| **Diagnosed by** | | | | | |
| Doctor | 10 (52.6) | 10 (100) | 31 (93.8) | 51 (82.3) | NA |
| NGO worker | 09 (47.4) | 00 (0.0) | 01 (3.1) | 10 (16.1) | |
| Neighborhood | 00 (0.0) | 00 (0.0) | 01 (3.1) | 01 (1.6) | |

*Student's T-test P>0.05 **Chi-square test P>0.05

NA=not applicable

### 3.2 Nutrient-rich Food Intake Per Day

About 37.1% of the cases and almost half (50.0%) of the controls ingested protein rich foods (P<0.05) per day. Lower consumption of protein-rich foods by cases was associated with severe arsenicosis while not severe (mild/moderate) cases took higher rate of protein foods than severe group (P<0.05). Similarly, lower consumption of both LVs (38.7%) and NLVs (37.1%) among cases (both severe and not-severe) were observed than control (LVs=56.7% and NLVs=53.3%) (Table 2).

### 3.3 Protective Role of Different Vegetables on Arsenic Induced Skin Lesions

Table 3 outlined that among all (n=17) LVs (n=08) and NLVs (n=09) most of the vegetables have significant (P<0.05) protective roles on arsenic induced-skin lesions in unadjusted model/or bivariate analysis (shown by crude odds ratio/COR) except green aurum leaves, jute leaves and tomatoes (P>0.05). However, only seven vegetables (two LVs and five NLVs) remained in the final adjusted model (shown by adjusted odd ratios/AOR) of multiple logistic regression (MLR). MLR model showed amongst all most frequently eaten vegetables (n=17) per day, *Momordica diocia* (spiny gourd) has the highest skin protective role on arsenicosis [AOR 8.2 95% CI (2.11-31.9), P=<0.01], followed by *Ipomoea acquatica* (Water spinach, AOR: 7.3), *Basella alba* (Indian spinach, AOR:6.2), *Solanum tuberosum* (Potato, AOR:4.0), *Vigna unguiculata sesquipedalis* (Snake/long bean,AOR:3.2), *Trichosanthes anguina* (Snake gourd,AOR:1.2) snd *Abelmoschus esculentus* (Ladies finger, AOR:1.2). This study reported not taking LVs like Indian spinach (64.5%) and water spinach (59.7%) among cases than controls (23.3% and 26.7%) are respectively 6.2 and 7.3-times (AOR) more likely to be associated with arsenicosis (P<0.01). Similarly, abstinence from taking some NLVs (e.g. spiny gourd, potato, snake gourd, long/snake bean and ladies finger) among cases than controls are respectively 8.2, 4.0, 1.2, 3.2 and 1.2-times (AORs) more likely to be associated with arsenicosis (P<0.05).

### 3.4 Influence of the Intake of Different Vegetables on the Severity of Arsenicosis

Table 4 showed severity of arsenicosis in terms of taking more/less vegetables between two groups (severe/not severe) having arsenicosis. Lower consumption of vegetables (both LVs and NLVs) was associated with severe arsenicosis while not severe (mild/moderate) cases took higher rate of both LVs and NLVs than severe group (P<0.05). Among leafy vegetables those were frequently consumed per day by the study subjects, two LVs (Indian spinach or *Basella alba* and Water spinach or *Ipomoea acquatica*) reportedly showed to be protective against severe arsenicosis (P<0.05). Similarly, five NLVs (Spiny gourd or *Momordica diocia*, Potato or *Solanum tuberosum*,

**Table 2.** Nutrient-rich food intake frequency per day between groups

| Frequency distribution of food intake | Total Cases (n=62) | Case n (%) | | | Control | Test- statistic |
|---|---|---|---|---|---|---|
| | | Classification of arsenicosis | | | | |
| | | Not severe (n=29) | | Severe | (n=60) | |
| | | Mild (n=19) | Moderate (n=10) | (n=33) | | |
| **Animal-protein-rich foods** | | | | | | |
| Yes | 23 (37.1) | 10 (52.6) | 05 (50.0) | 09 (27.3) | 30 (50.0) | **P*** |
| No | 39 (62.9) | 09 (47.4) | 05 (50.0) | 16 (72.7) | 30 (50.0) | |
| P-value | P* | | | | | |
| **Mineral and vitamin-rich foods** | | | | | | |
| ***Leafy vegetable/LVs (n=08)*** | | | | | | |
| Yes | 24 (38.7) | 07 (21.1) | 05 (40.0) | 12 (54.5) | 34 (56.7) | P* |
| No | 38 (61.3) | 12 (78.9) | 05 (80.0) | 15 (45.5) | 26 (43.3) | |
| P-value | P* | | | | | |
| ***Non-leafy vegetables/NLVs (n=09)*** | | | | | | |
| Yes | 23 (37.1) | 08 (42.1) | 04 (40.0) | 11 (33.3) | 32 (53.3) | P* |
| No | 41 (62.9) | 11 (57.9) | 06 (60.0) | 22 (66.7) | 28 (46.7) | |
| P-value | P* | | | | | |

*Chi-square/Fisher's Exact test and P<0.05

**Table 3.** Protective role of different vegetables on arsenic induced skin lesions

| *Leafy vegetables* | | Case (n=62) | Control (n=60) | COR (95% CI) | (B) | AOR (95% CI) |
|---|---|---|---|---|---|---|
| *Basella alba* (Indian spinach) | Intake^R | 22 (35.5) | 46 (76.7) | 1 | | |
| | No | 40 (64.5) | 14 (23.3) | 4.5(1.7-12.2)** | B=1.839 | 6.2 (1.58-24.9)** |
| *Amaranthus tricolor* (Joseph's coat) | Intake^R | 04 (6.5) | 06 (10.0) | 1 | | |
| | No | 58 (93.5) | 54 (90.0) | 2.39 (7.53-7.5)* | | |
| *Amaranthus dubius* (Amaranth) | Intake^R | 06 (9.7) | 04 (6.7) | 1 | | |
| | No | 56 (90.3) | 56 (93.3) | 2.28 (.918-5.7)* | | |
| *Cucurbita maxima* (Pumpkin leaves) | Intake^R | 32 (51.5) | 24 (40.0) | 1 | | |
| | No | 30 (48.5) | 36 (60.0) | 2.00 (.80-4.9)* | | |
| *Ipomoea acquatica* (Water spinach) | Intake^R | 25 (40.3) | 50 (73.3) | 1 | | |
| | No | 37(59.7) | 10 (26.7) | 4.1 (1.6-10.6)** | B=1.984 | 7.3 (1.91-27.6)** |
| *Colocasia antiquorum* (green aurum leaves) | Intake^R | 29 (46.7) | 26 (43.3) | 1 | | |
| | No | 33 (53.3) | 34 (56.7) | 1.82 (.75-4.4) | | |
| *Nasturtium officinale* (Water cress) | Intake^R | 04 (6.5) | 16 (13.3) | 1 | | |
| | No | 58 (93.5) | 52 (86.7) | 2.14 (.73-6.3)* | | |
| *Corchorus capsularis* (Jute leaves) | Intake^R | 23(37.1) | 18 (30.0) | 1 | | |
| | No | 39 (62.9) | 42 (70.0) | 1.62 (.66-3.9) | | |
| Non-Leafy vegetables | | | | | | |
| *Cucurbita maxima* (Pumpkin) | Intake^R | 37 (59.7) | 46 (76.6) | 1 | | |
| | No | 25 (50.3) | 14 (23.4) | 2.22 (.83-5.95)* | | |
| *Momordica charantia* (Bitter gourd) | Intake^R | 29 (46.8) | 38 (63.3) | 1 | | |
| | No | 33 (53.2) | 22 (36.7) | 1.96 (.80-4.8)* | | |
| *Solanum lycopersicum* (Tomato) | Intake^R | 08 (12.9) | 10 (16.6) | 1 | | |
| | No | 54 (87.1) | 50 (83.4) | 1.17 (.36-3.9) | | |
| *Momordica diocia* (Spiny gourd) | Intake^R | 24 (38.7) | 34 (56.7) | 1 | | |
| | No | 38 (61.3) | 26 (43.3) | 4.48 (1.76-11.4)** | B=2.107 | 8.2 (2.11-31.9)** |
| *Daucus carota* (Carrot) | Intake^R | 03 (4.8) | 08 (13.3) | 1 | | |
| | No | 59 (95.2) | 52 (86.7) | 3.03 (.63-14.5)* | | |
| *Solanum tuberosum* (Potato) | Intake^R | 37 (59.7) | 52 (86.7) | 1 | | |
| | No | 25 (40.3) | 08 (13.3) | 4.22 (1.67-10.6)** | B=1.404 | 4.0 (1.12-14.7)* |
| *Vigna unguiculata sesquipedalis* (Snake/long bean) | Intake^R | 23(37.1) | 42 (70.0) | 1 | | |
| | No | 39 (62.9) | 18 (30.0) | 3.08 (1.15-8.2)* | B=1.177 | 3.2 (.87-12.1)* |
| *Trichosanthes anguina* (Snake gourd) | Intake^R | 24 (38.7) | 54 (90.0) | 1 | | |
| | No | 38 (61.3) | 06 (10.0) | 3.29 (1.31-8.2)* | B=.197 | 1.2 (.22-6.7)* |
| *Abelmoschus esculentus* (Ladies finger) | Intake^R | 33 (53.2) | 50 (83.3) | 1 | | |
| | No | 29 (46.8) | 10 (16.7) | 2.38 (.97-5.8)* | B=.222 | 1.2 (.27-5.9)* |

Overall percentage 81.5%, -2LL =71.425, Nagelkerke R Square=.537, Model co-efficient (Chi-square)= 44.748, degree of freedom/ df=5, P=0.000, Hosmer Lemeshow goodness of fit=5.716, P=.679, Crude odds ratio=COR, Beta coefficient=B, Adjusted Odds ratio=AOR, ^R Reference category, *P<0.05, **P<0.01

Snake/long bean or *Vigna unguiculata sesquipedalis*, Snake gourd or *Trichosanthes anguina* and ladies finger or *Abelmoschus esculentus*) have been showed protective effect against the development of severe arsenic-induced skin lesion (P<0.05).

## 4. Discussion

This study showed protective roles of some leafy and non-leafy vegetables on arsenic-induced skin lesion among women in rural Bangladesh and less intake-frequency of these vegetables also reported to influence on the severity of the skin lesion. The positive association of some leafy and non-leafy vegetables with the extent of arsenic related skin lesions can be explained by the facts that vegetables are one of the richest natural sources of vitamins (especially antioxidant vitamin A, C, E, and B-complex) and minerals (iron and antioxidant copper, zinc, and selenium) which have concomitant roles of both essential nutritive activities and anti-oxidants capacity, have been shown to exert protective effects on body-burden of arsenic. Previous study [18] also reported intake of different gourds, roots (NLVs) and protein inversely

**Table 4.** Influence of the intake of different vegetables on the severity of arsenicosis

| **Leafy vegetables** | | **Total (n=62) n (%)** | **Having skin lesions** n (%) | | |
|---|---|---|---|---|---|
| | | | **Severe (n=33)** | **Not severe (n=29)** | **P-value@** |
| Indian spinach | Intake | 41 (66.1) | 14 (42.4) | 27 (93.1) | P=0.005** |
| | No | 21 (33.9) | 19 (57.6) | 02 (06.9) | |
| Joseph's coat | Intake | 04 (6.5) | 01 (3.0) | 03 (10.3) | P=0.332 |
| | No | 58 (93.5) | 32 (97.0) | 26 (89.7) | |
| Amaranth | Intake | 06 (9.7) | 01 (3.0) | 05 (17.2) | P=0.089 |
| | No | 56 (90.3) | 32 (97.0) | 24 (82.8) | |
| Pumpkin leaves | Intake | 32 (51.5) | 17 (51.5) | 15 (51.7) | P=0.987 |
| | No | 30 (48.5) | 16 (48.5) | 14 (48.3) | |
| Water spinach | Intake | 25 (40.3) | 09 (27.3) | 16 (55.2) | P=0.025* |
| | No | 37 (59.7) | 24 (72.7) | 13 (44.8) | |
| Green aurum leaves | Intake | 29 (46.8) | 17 (51.5) | 12 (41.4) | P=0.425 |
| | No | 33(53.2) | 16 (8.5) | 17 (58.6) | |
| Water cress | Intake | 04 (6.5) | 03 (9.1) | 01 (3.4) | P=0.616 |
| | No | 58 (93.5) | 30 (90.9) | 28 (96.6) | |
| Jute leaves | Intake | 23 (37.1) | 10 (30.3) | 13 (44.8) | P=0.237 |
| | No | 39 (62.9) | 13 (69.7) | 16 (55.2) | |
| **Non-leafy vegetables** | | | | | |
| Pumpkin | Intake | 37 (59.7) | 18 (54.5) | 19 (65.5) | P=0.380 |
| | No | 25 (40.3) | 15 (45.5) | 10 (34.5) | |
| Bitter gourd | Intake | 29 (46.8) | 13 (39.4) | 16 (55.2) | P=0.214 |
| | No | 33 (55.2) | 20 (60.6) | 13 (44.8) | |
| Tomato | Intake | 08 (12.9) | 03 (9.1) | 05 (17.2) | P=0.456 |
| | No | 54 (87.1) | 30 (90.9) | 24 (82.8) | |
| Spiny gourd | Intake | 24 (38.7) | 06 (18.2) | 18 (62.1) | P=0.004** |
| | No | 38 (61.3) | 27 (81.8) | 11 (37.9) | |
| Carrot | Intake | 03 (4.8) | 00 (0.0) | 03 (10.3) | P=0.097 |
| | No | 59 (95.2) | 33 (100.0) | 26 (89.7) | |
| Potato | Intake | 37 (59.7) | 14 (42.4) | 23 (79.3) | P=0.003** |
| | No | 25 (40.3) | 19 (57.6) | 06 (20.7) | |
| Snake/long/Asparagus bean | Intake | 23 (37.1) | 07 (21.2) | 16 (55.2) | P=0.006** |
| | No | 39 (62.9) | 26 (78.8) | 13 (44.8) | |
| Snake gourd | Intake | 24 (38.7) | 08 (24.2) | 16 (55.2) | P=0.013* |
| | No | 38 (61.3) | 25 (75.8) | 13 (44.8) | |
| Ladies finger | Intake | 33 (53.2) | 12 (36.4) | 21 (72.4) | P=0.005** |
| | No | 29 (46.8) | 21 (63.6) | 08 (27.6) | |

@Chi-square test/Fisher's Exact Test; Not severe=mild/moderate arsenicosis; *P<0.05, **P<0.01

associated with arsenic-induced skin lesion risk among male and female cohort in Bangladesh.

Abstinence from the dietary intake of leafy/non-leafy vegetables among cases than controls is (P<0.05) more likely to be associated with arsenicosis. On the other hand, Consuming more leafy vegetables like 'Indian spinach (*Basella alba*)' and 'water spinach (*Ipomoea acquatica*)' and non-leafy vegetables like 'spiny gourd (*Momordica diocia*)', 'Potato (*Solanum tuberosum*)', 'snake gourd (*Trichosanthes anguina*)', 'snake/long bean (*Vigna unguiculata sesquipedalis*)' and 'Ladies finger (*Abelmoschus esculentus*)' were found to have less severe arsenic-induced skin lesions (mild/moderate) than severe one. This may be due to having intrinsic nutrient potentials and presence of other non-nutritive bioactive compounds and phytochemical characteristics of different leafy and non-leafy vegetables as reported by the most of the prior and recent studies [11,12,19-26,27-31,33,37]. As mentioned earlier vegetables are important for human nutrition in terms of bioactive nutrient molecules (dietary fiber, antioxidant pro-vitamin A/or Carotenoids, vitamin C, vitamin E and minerals e.g. copper, zinc, selenium) and non-nutritive phytochemicals (phenolic compounds, flavonoids, saponin, bioactive peptides, glycoside etc.). These nutrient and non-nutrient molecules reduce the risk of non-communicable diseases [11,37]. Medicinal plants [9,10,19-21,33,38] those having substantial amount of nutrients and non-nutrient poly-phenolic components (plant's secondary metabolites) have potent abilities to act as antioxidant, antibacterial and anti-inflammatory agents which accelerate the rapid healing of damaged skin tissue at various stages of wound healing (i.e., collagenation, wound closure and epithelialization) in addition to reducing damage caused by oxygen radicals in the area of dermal injury [19]. Moreover, antioxidant fights against free radicals and exert their action either by scavenging the reactive oxygen species or protecting the oxidant defense mechanism. Thus, presence of different nutrients and non-nutrients in LVs and NLVs would offer a synergistic effects to ensure the protection against arsenic-induced skin lesion among women.

In this study, dietary intake of two LVs (*B. alba and I. acquatica*) were found to be negatively associated with arsenicosis and not consuming these two LVs, the probability of infecting arsenic-induced skin lesion increased respectively 6.2 and 7.3-folds among cases than controls (P<0.01). This might be due to arsenic is excreted following methylation reactions, which are mediated by folate [1] and LVs are excellent, richest source of folate, thus folic acid with other bioactive polyphenols could facilitate arsenic methylation and excretion, performed antioxidant and free radical scavenging activity thereby reducing arsenic toxicity and skin lesions. Studies reported *B. alba* has great ethno-medicinal and nutritional importance as the plant is rich in vitamin A (carotenoids), vitamin C, organic acids and many amino acids along with active constituents like basella saponin, kaempherol, betalain, flavonoids, saponins, peptide and phenolic compounds which are therapeutically used for antioxidant, anti-inflammatory, antiulcer, antifungal, anticonvulsant, analgesic activities and for the treatment of skin diseases and many more[22,26,27,30]. Similarly, higher intake (93.1%) of *I. acquatica* by mild/moderate arsenicosis group than that of severe (42.4%) was significantly associated with the degree of severity of arsenicosis. This might be due to the prophylactic effect of ascorbic acid [39] and anti-apoptotic potential of both phenolic and flavonoids against arsenic-toxicity through antioxidant mechanism [23,25,31]. Studies reported abundant presence of different nutrients (e.g. ß-carotenes and ascorbic acid), and non-nutrients (phenolics: gallic acid and chlorogenic acid; flavonoids compounds: myricetin, quercetin and apigenin) in *I. aquatica* would play crucial role in overall protective and cumulative effect against arsnic-induced skin lesion [23,31]. Moreover, study reported phenolics and saponins exhibited chelating properties to mop up arsenites from the body [25].

This study showed dietary intake of *Momordica diocia* (spiny gourd) has 88% (inverting odds ratio 8.2) averting role in occurring arsenicosis which means controls are less likely to develop arsenicosis than cases due to more ingestion of spiny gourd and has the highest skin protective role among all vegetables, followed by other NLVs like *Solanum tuberosum (75%), Vigna unguiculata sesquipedalis (69%), Trichosanthes anguina (17%)* and *Abelmoschus esculentus (17%).* This might be due to the nutrient contents of ascorbic acid, the ratio of unsaturated fatty acid of spiny gourd [29], and antioxidant, antibacterial, anti-inflammatory, antimicrobial and anti-allergic agents of most of the medicinal plants due to possessing bioactive compounds and the phytochemical substances (poly phenols) as reported most of the studies [19-21,33]. Study on animal model also reported aqueous extract of spiny gourd flesh might have suppressive effect, therapeutic potential for allergic responses (by inhibiting histamine release and decreased IgE levels) in vitro and in vivo as compared to control and alleviated development of atopic dermatitis-like skin lesions [29]. Additionally, considering the diversity of the phyto-constituents and bioactive metabolites (gallic acid, chlorogenic acid, polyphenols, flavonoids like Rutin, alkaloids like Solanine) present in *S. tuberosum*, studies reported to promote the wound healing process by increasing epithelialization rate and collagen synthesis and subsequent decreasing the overproduction of free radicals, facilitating oxygen diffusion mainly due to potatoes antioxidant, antibacterial capabilities. Thus, studies reported varieties of *S. tubersome* contain anthocyanin which suppress the modulation of atopic dermatitis[40] and can heal wound in experimental mice [24].

Studies outlined high nutritional contents (proteins, carbohydrates, calories, dietary fiber trace elements and macro-minerals) and presence of different bioactive compounds (polyols, phytosterols, phenolics, neochlorogenic acid, chlorogenic acid, caffeic acids and saponins) in cowpea/asparagus/snake bean's seeds and pods [41-44]. Thus having rich contents of both nutrient and non-nutrient substances, in this study snake bean (*V. unguiculata sesquipedalis)* showed protective role against arsenic-induced skin lesion through performing its antioxidant (radical scavenging activity), antibacterial, anti-inflammatory, and anti-allergic activities [42-44]. Moreover, another NLV snake gourd (*T. anguina)* is a good source of Vitamin A, Vitamin B (mainly niacin), Vitamin C and minerals (magnesium, calcium, potassium, phosphorus, iron). The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, glycosides, saponins phenolic acids which makes the plant pharmacologically and therapeutically active [32,45].

Like this study, okra (*Abelmoschus Esculentus*) reported to have potential dietary medicine with nutraceutical importance against gastric lesions in mice model [28]. Okra has intrinsic active nutritional values (Vitamin A, K and C, B-vitamins (B1, B2, B6, folic acid), linoleic acid, polysaccharides, calcium, iron, manganese, and magnesium) along with huge nutraceutical (antioxidant, immunomodulatory, Antiproliferative and proapoptotic) and therapeutic potentials (antibacterial, anticancer, antidiabetic, lipid lowering agents) for having bioactive components (phytochemicals e.g. poly phenols like catechin, flavonoids (quercetin), Lectins, Pectins, Glycosylated compounds) [12,14,28,46,47].

## 5. Conclusions

This study showed the protective and beneficial roles of some commonly eaten vegetables against both development and severity of arsenic-induced skin lesions. Large samples longitudinal study in this important field of therapeutic intervention is warranted.

### Strength and limitation of the study

This case-control was conducted to assess the role of dietary intake of some 'vegetables' on arsenic-induced skin-lesions. Both qualitative and quantitative approaches (one 24-hour recall, food frequency/week and food history-record/week) were applied to measure habitual dietary practices in terms of taking leafy and non-leafy vegetables. Multiple logistic regression (MLR) analyses were performed to find out protective roles of some leafy (n=08) and non-leafy (n=09) vegetables on arsenic induced skin-lesions (arsenicosis) among rural women while adjusting demographic variables. Moreover, how dietary intake frequencies of vegetables influence the degree of severity on arsenicosis among village women also determined. However, recall bias- the main limitation of case-control study cannot be ruled out, and lack of information on sources of water for irrigation and arsenic contents of foods of those particular areas and not including males in the study- were other limitations. Despite some limitations this study has distinct strengths, most of the studies performed using animal model to see the phytochemical properties of plants, or association of nutrients with non-communicable diseases (NCDs), only few studies have evaluated the impact of vegetables on arsenic-associated skin lesion. In particular, this study helps to fill an important research gap to find out the strength of relationship about the protective roles of some leafy and non-leafy vegetables with the arsenic induced skin-lesions.

## Author Contributions

Nasrin S, Islam SN, Kawser M, and Ahmed S conceptualized the Study and Islam SN was the lead supervisor, field supervisions and medical supports were provided by Saha AK and Haque A while field supervisions at different stages were accomplished by Rahman R and Akter A. Methodology was developed by Nasrin S, Islam SN and Kawser M. Field training given to the staffs and acquisition of data by Nasrin S, Rahman R and Akter A. Statistical data were analyzed and interpreted by Kawser M. Literature Reviewed by Kawser M, Rahman R, Akter A and Nasrin S. Nasrin S and Kawser M prepared the original draft of the manuscript. Islam SN, Ahmed S, Saha AK and Haque A had significant contribution for critical intellectual contents. All authors read, revised, edited and approved the final manuscript.

## Conflicts of Interest

The authors declare no potential conflicts of interest regarding the publication of this paper.

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ARTICLE

# Wild Edible Plants Used by the Tribes of Panvel and Uran Tahsils in Alibaugh District, India: Ethnobotanical Application and Tribal Recipes

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ABSTRACT

The "Indus-Vedic" cultural heritage of India is well-known. Wild edible plants, sometimes known as weeds, are widely consumed in India's varied areas. Wild edible plants and weeds are essential for tribes' survival, both as a source of food and as a source of money, such as timber. This study aims to identify wild vegetables collected for ethnomedical purposes and their recipes by the local people, as well as determine the local uses and names of these plants, with the goal of closing the gap in traditional knowledge regarding the utility of wild plant species and tapping the hidden potential resources for proper utilization, exploitation, and nutritive evaluation. A field research study was conducted two years 2020-2021. 34 wild vegetable plant specimens were collected during this time. The names of the plants found in the area, as well as the parts that were used and how they were prepared, were examined and recorded. This type of extensive survey technique could assist aspiring scientists in learning about the health advantages of wild food plants and weeds, which can subsequently be combined to generate successful crop plants. Such a system will benefit in the mitigation of food shortages, the regeneration of infertile lands, and the enhancement of rural economies.

## 1. Introduction

Tribes' dependence on unconventional wild vegetables is because of their habitat in hilly areas surrounded by forest. The majority of their agricultural fields are on hills and are therefore less productive. The scarcity of cereals and pulses begins with the arrival of winter. Tribes go to the nearby forest on a regular basis to obtain forest produce to augment their nutrition. To fulfil their hunger, the tribals are constantly on the lookout for unusual wild foods that have been growing for years [1].

For the uncommon wild vegetables, the monsoon is the most abundant season. These vegetables are abundant from July to September in forests, along hill slopes, nearby river banks, around ponds, and in and around their hamlets where abundance of cow dung is available for their good growth. The majority of wild vegetables are accessible for good growth throughout the first two

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months of the monsoon. Though the majority of wild vegetables are available to tribals during the first two months of the monsoon, just a few are left behind for their family members due to strong demand in the local taluka market. Because of their ethnomedicinal value, the unusual wild vegetable brings in extra money for the tribals.

Though functional food is higher in minerals, proteins, and beta-carotene than unusual green leafy vegetables, tribals' intake of functional food is lower due to non-availability in bulk, improper cooking methods, and an excess of crude fibre; thus, tribals exhibit signs and symptoms of malnutrition, with growing children and lactating mothers being the main victims [2]. Fruits contribute more to tribal diets than cereals, however not to the extent recommended by the ICMR, which could be one of the causes of vitamin A deficiency among them.

The diets of diverse Indian tribal tribes were found to be seasonal, lacking in green vegetables, and high in crude fibre, according to studies [3]. Children, breastfeeding moms, and pregnant women are the most susceptible to such imbalanced nutrition. During the monsoon season, infectious disorders linked to malnutrition are especially common among tribals. In this season, tribal people's diets contain only a small amount of leafy vegetables, despite the fact that green leafy vegetables are readily available [4]. Fibre is abundant in a variety of wild vegetables. The primary impediment to the biological availability of proteins and vitamins is fibre. The oil is not included in the diet of the tribes. Milk and milk products are completely absent from their everyday dietary intake.

Several research on wild edible plants used by various groups in India have been undertaken, including edible plants from Maharashtra's Melghat forest [5], plant resources from Maharashtra's Thane area [6], and wild edible plants from India's Arunachal Pradesh [7], wild edible plants from Annamalai [8], Coimbatore district, Western Ghat [9], Sikkim Himalayan plant dietary applications [10,11]. Tripura, India and Ahmednagar, Maharashtra [12], biodiversity in Konkan Wild Vegetables [13,14]. Rural people eat wild edible plants as vegetables. Several investigations on traditionally used herbal remedies have been undertaken in Maharashtra (especially on the Western Ghat). In the Panvel and Uran regions, no effort had been done to document the diversity and utility of wild vegetables. As a result, the current research was designed to describe the variety of wild vegetables consumed by rural and urban residents in the Panvel and Uran areas of Albaugh, Maharashtra, India.

## 2. Materials and Methods

### Selection of the site

Panvel and Uran are the most economically underdeveloped places since their human habitats are inaccessible to modern culture and civilization. The tribals in this area are still surrounded in a past era, without access to all of the modern amenities. Their anthropological dictionary is totally lacking of the magical word "science". For our research, we chose a rural location in Panvel and Uran taluka.

### Collection of Wild Vegetables

Vegetable samples were collected on a quarterly basis in the study locations. In the Panvel and Nerul Navi Mumbai areas, vegetable samples were also collected from village elders, farmers, vegetable merchants, customers, and vegetable middlemen.

### Plant Identification

Specimens were identified using standard procedures [15,16] and Flora of Gujrat) with supporting information for ethnomedical uses and recipes [17-19]. Tribes were interviewed to learn about local vegetable names, habits, habitats, cooking methods, ingredients, current availability, distance travelled to acquire the vegetables, time gap before cooking, and quantity used in preparation.

## 3. Results and Discussion

Tribes consume largely leafy vegetables that grow like weeds in the wild [20]. According to the findings of this research, the Panvel and Uran areas are rich in wild vegetable species with enormous potential for human use, contributing favourably to the local economy and livelihoods of the many populations. In this work, a total of 34 wild edible plant species from 22 families and 29 genera have been found, classified, and discussed. Plant species' botanical names, as well as local names, habits, families, parts used, modes of usage, ethnomedicinal applications, and tribal recipes, are arranged alphabetically. With five species, the Cucurbitaceae family takes the lead, followed by Araceae with four species, the Yam family or Dioscoreaceae with three species, Vitaceae, Fabaceae, and Chenopodiaceae with two species respectively, and the rest with one species each.

The leaves, which represent 18 species, were found to be the most consumed part of the plant, followed by fruit (15 species), bulb/rhizome/corm/tuber (08 species), tender shoots (06 species), flowers/inflorescence (06 species), and seeds (02 species). Wild edible plants found in the research area were frequently used as vegetables in curry, soups, and pickles. Many of them are utilized for healing purposes due to their ability to cure a variety of diseases. Some are eaten raw, while others are cooked, and many are used as food substitutes when food is scarce.

According to the findings of this study,98 percent of all plants documented have medicinal properties and are commonly used for common ailments such as coughs, asthma, stomach issues, skin infections, urine, and so on. According to the market survey, a total of 34 wild edible plants have commercial value among locals and are frequently sold in small markets or even transported to larger marketplaces nearby. The market price of these plants has been compared between the remote Uran market and Navi Mumbai town, and it has been discovered that the selling price of these plants doubles to triples once they reach urban areas. Some of these wild edible plants are isolated to rural populations and are not very popular in urban markets, according to reports.

1) *Alylosia lineta* Wight and Arn's. (Local Name: Jangli Tur Sheng, Family-Fabaceae).

**Edible parts**: Fresh leaves, young pods, and seeds are all edible portions.

**Tribal recipe**: young leaves are utilised as vegetables in this traditional dish. Salt is added to mature pods before they are boiled. The seeds are removed and consumed. Young pods are eaten directly.

**Ethnomedicinal applications:** Its ethnomedical uses include itching relief and the treatment of blood impurities. The malnourished child is given plain boiled seeds.

2) *Amaranthus gangeticus* (Local Name-Ran Math, Family-Amaranthaceae).

**Edible parts**: young leaves and tender shoots are edible parts.

**Tribal recipe**: Fresh young leaves and stem portions are boiled and served in curries or as a vegetable with a pinch of salt and chilies in a tribal dish.

**Ethnomedicinal applications:** Applications include wound healing, cough relief, and reducing the effects of alcohol.

3) *Amorphophallus commutatus* (Local Name: Shevli, Family: Araceae).

**Edible Parts**: All parts of the plant are edible, including tender shoots, leaves, flowers, and fruits.

**Tribal recipe**- Open inflorescence is fried on a light flame and served as a vegetable in a tribal dish. The leaves are cooked in water before being eaten.

**Ethnomedicinal applications:** Infusion is used to treat stomach issues in traditional medicine.

4) *Amorphophallus sylvaticus*, Roxb. Kunth. (Local name: Jangli Suran, Family- Araceae).

**Edible parts**- Corm, tender petiole, and young leaf are all edible components.

**Tribal recipe**- The corm is edible after being washed and boiled for a long time. Cut fresh young petioles into pieces, boil them, and eat them. Chopped young leaves are used as a vegetable.

**Ethnomedicinal applications:** Piles, coughing, and asthma are all treated with this herb.

5) *Atriplex hortensis var. hortensis*, (Local name: Chandan Batwa or Ran Batwa. *Family* Chenopodiaceae).

**Edible part**-The leaves are edible.

**Tribal recipe**-Cooked and consumed like a vegetable, the fresh leaves are used. Ingredients include salt and chilies.

**Ethnomedicinal applications:** A decoction of the leaves is used as a tonic, providing energy to weak patients. Lactating women should consume the vegetable to increase the amount of breast milk they produce.

6) *Bambusa arundinacea* Linn. (Local Name: Bamboo Comb), Family: Gramineae (Poaceae)

**Edible parts-**Part-tender shoots that are edible

**Tribal recipe**-The combs are peeled and sliced into little pieces, then heated in boiling water for a while before being thrown in with water. Bamboo is boiled and eaten with salt, chili and peppers.

**Ethnomedicinal applications:** Bambusa leaves, shoot and seeds used as astringent and laxative.

7) *Brassica nigra Linn.* (Local Name: Rai, Family-Cruciferae/Brassiceae).

**Edible par**t- The leaves are edible.

**Tribal recipe-** Fresh leaves are cooked with onions and eaten as part of a tribal dish. Salt and chillies are the major ingredients.

**Ethnomedicinal applications:** Intestinal worms, skin infections, indigestion, stomach pain, and swellings are all treated with this herb.

8) *Capparis moonii* Wight. (Local Name-Pendra), Family-Capparaceae)

**Edible part**-Fruit

**Tribal recipe**- The fruits are sliced and the seeds are removed in this tribal dish. With some oil, chilies, and salt, the sliced pieces are fried on a low burner.

**Ethnomedicinal applications:** Seed paste is used as an antibacterial on wounds in traditional medicine. Also known as Anasarca.

9) *Chenopodium album* (Local Name: Chakwat, Family-Chenopodiaceae).

**Edible part**: Leaves

**Tribal recipe**- Fresh leaves are cooked with salt and chiles as a vegetable.

**Ethnomedicinal applications:** Used to treat throat infections. After the symptoms of chickenpox appear, that plant's juice is mixed with honey and used to make the person suffer vomiting.

10) *Colocasia antiquorum* L. (Local Name-Arve, Family-Araceae).

**Edible parts**: Tubers

**Tribal recipe**- The tubers are pilled and cut into pieces, then washed in water and cooked as a vegetable; they can also be eaten raw after washing and roasting in hot ash, or cooked with oil, salt, and chilies and then consumed.

**Ethnomedicinal applications:** It has digestive qualities.

11) *Colocasia esculenta* (Local Name: Alu, Ghuiyan, Family: Araceae).

**Edible parts**: Leaves with a long succulent petiole and rhizome are edible.

**Tribal recipe-**Rhizomes are washed often, then cooked and eaten with salt and chiles after the skin has been removed.

**Ethnomedicinal applications:** Astringent, stimulant, and rubefacient effects are found in the leaf juice. Corm juice is a laxative that can also be used to reduce bleeding in piles. It is used to help breastfeeding women produce more milk.

12) *Cucurbita maxima* (Local Name: Danger, Family-Cucurbitaceae).

**Edible parts**-Tender shoots, leaves, flowers, and fruits are all edible components of the plant.

**Tribal recipe**-Open flowers are fried on a low flame and used as vegetables in a tribal dish. Cut the leaves into pieces, boil them in water, and eat them.

**Ethnomedicinal applications:** Flowers are used as an ethnomedicinal treatment for skin infections.

13) *Dioscoria pentaphylla (Local Name: Gabholi, Family-Dioscoreaceae).*

**Edible parts**-Flowers in inflorescence form are edible.

**Tribal recipe**- When male flowers are green, they are consumed, while female flowers are cream-colored. Male and female plant flower buds are used in curries as vegetables. Flower buds that have been cooked are delicious popular food delicious.

**Ethnomedicinal applications:** Aphrodisiac, it is said that eating a lot of flowers and vegetables can make you more attractive to other people.

14) *Dioscorea bulbifera (Local name-Kadukand, family- Dioscoreaceae).*

**Edible part-** The underground tubers are known as "kadukand" and are edible.

**Tribal recipe**- Underground kadukand can be made edible by treating it as follows: It is cleansed initially by removing the root hairs. They are properly washed in running water, peeled, and sliced into slices before being served with salt. They can be placed in hot ash for 2 to 3 hours before being cut into pieces and fried. Bulbul's vegetables are prepared in a manner that is comparable to that of potato tubers. The outside warty layer is removed first, and the inside section is used to prepare vegetables. It can also be roasted and eaten with salt in hot ash.

**Ethnomedicinal applications:** Ulcers, piles, diarrhea, and syphilis are all treated with dried and powdered tubers. The sedative qualities of young bulbils are well-known.

15) *Dioscorea oppositifolia* L. (Local Name Dukkar-Paspoli, Family-Dioscoreaceae).

**Edible parts**-Underground tubers, flowers, and aerial bulbils are all edible.

**Tribal recipe**-Tubers are edible after several items of washing and boiling, and long-duration leaves are consumed when food is scarce. Bulbils are boiled after turning brown-black, the outer wart removed, and the interior starch consumed. Bulbils are also roasted and consumed after the outer coating has been removed.

**Ethnomedicinal applications:** The use of flowers is common. It promotes sexual desire in the diet. It's used to cure piles and stomach pain, among other things.

16) *Garuga pinnata* Roxb. (Local Name- Kakad, Family-Burseraceae).

**Edible part**: Fruits

**Tribal recipe**: the drupes can be eaten fresh, boiled, or pickled. They are highly acidic and have a cooling effect on the digestive system. To neutralize the raphides, fruits were combined with shevli during vegetable preparation.

**Ethnomedicinal applications:** The juice of the stem is used as a treatment for conjunctivitis opacity. Asthma can be helped by mixing leaf juice with honey.

17) *Guizotia abyssynica* Cass. (Local Name-Karale, Family-Compositae).

**Edible part**-Leaves

**Tribal recipe**- The leaves are finely chopped and fried on a low temperature, with salt and chillies added.

**Ethnomedicinal applications:** The oil is derived from seeds and has ethnomedicinal applications. It has a laxative effect. It can also be used on a person who is suffering from joint discomfort or Rheumatism. Ginger and castor oil are tainted with it.

18) *Hibiscus cannabis Linn*. (Local name: Ambadi.) Family Malvaceae

Edible part-Leaves

**Tribal recipe**-Fresh leaves are cooked with salt and chilies and consumed as a vegetable in this tribal dish.

**Ethnomedicinal applications:** cooked leaves with rice are given to people who have dyspepsia caused by

Mahuwa oil. It's a pain reliever. If there is a problem passing urine, a decoction of seeds is given.

19) *Holarrhena antidysentrica* Wall. (Local Name-Kuda Sheng), Family-Apocynaceae).

**Edible part:** Pods

**Tribal recipe-**The pods are fine chopped in this tribal dish. These pieces are rinsed and cooked in a mild amount of oil with salt and chiles. It was prepared over low heat.

**Ethnomedicinal applications**-Alkaloids with therapeutic characteristics are abundant in plant parts, especially fruits. Roots and bark are used to cure diarrhea since they are anti dysenteric drugs. It's acidic, and it's good for digestion and cooling.

20) *Leea indica* Burm (Local Name-Bhane, Family-Vitaceae).

**Edible part**-young leaves and stem

**Tribal recipe**-There are two different types of tribal recipes.

• Pilled and chopped into small pieces, the young stem is boiled in water and eaten with salt. • The water is discarded, and the young stem is pilled and chopped, along with the leaves, and cooked on a low flame, sometimes with crabs.

**Ethnomedicinal applications:** The stem juice is used as an anthelmintic, and the tuber is used as an antiseptic for wound care.

21) *Leea macrophylla* Roxb. (Local Name- Dinda, Family-Vitaceae).

**Edible part-**The tender leaves and fruits

**Tribal recipe**-Fresh leaves and fruits are cooked with salt and chiles as a vegetable.

**Ethnomedicinal applications:** The root tubers are astringent and also have ethnomedicinal applications. Because of its healing properties, mucilage is applied to wounds and sores.

22) *Luffa tuberosa* Roxb. (Local Name-Satpute, Family-Cucurbitaceae).

**Edible part**-Fruits

**Tribal recipe**-The fruits are cut open and the seeds are removed in this tribal dish. Then it was sliced, salted, and set aside for a while. Then they were rinsed and cooked on low heat.

**Ethnomedicinal applications:** a decoction of the roots is used as a stomachic medicine. The seeds are crushed into a paste and applied to swollen areas of the body.

23) *Madhuca longifolia* Linn. (Local Name-Mahuwa, Mowa, Tode, Family -Sapotaceae).

**Edible parts:** Flowers, seeds and fruits

**Tribal recipe**-Flowers and fruits are lightly cooked and eaten with salt in a tribal recipe. Seeds that have been extracted for oil can also be eaten.

**Ethnomedicinal applications:** Madhuca leaves are effective in the treatment of Eczema. Tree flowers help to breastfeed women produce more milk. Snake poisoning is treated with seeds. Seeds are used to extract oil, which is then consumed. Flowers are combined with putrefied jaggery to make Mahuwa liquor, a fermented wine.

24) *Manihot esculana* Pohl (Local Name: Tapioca, Family- Euphorbiaceae).

**Edible part:** Tuberose roots

**Tribal recipe**-Tapioca roots are consumed after boiling and roasting in a tribal recipe. Root flour meal is consumed after it has been boiled or roasted.

**Ethnomedicinal applications:** If you have a constipation problem, use it as an appetizer. Wounds and ulcers are also treated with this treatment. In dyspepsia and anorexia, it is used as an aperient.

25) *Melothria heterophylaa* Cogniaux (Local Name-Gomati, Family-Cucurbitaceae).

**Edible part**-Fruit

**Tribal recipe**-The fruits are chopped into little pieces, sprinkled with salt, and left for a few minutes in this tribal recipe. Then they're rinsed and fried in a small amount of oil on a low flame.

**Ethnomedicinal applications:** Its juice is used to treat stomach pain in folk medicine. Carminative

**Social Application**-A person whose parents have passed away is not permitted to eat the vegetable.

26) *Momordica dioca* Roxb. (Local Name-Kartoli, Family-Cucurbitaceae).

**Edible parts**- The immature fruits, as well as young leaves and tubers, are edible.

**Tribal recipe**-In urban areas, young fruits are in high demand. Curries with young green fruits are tribal recipe.

**Ethnomedicinal applications:** Tuberose roots are used in traditional medicine to treat bleeding piles, digestive and urinary problems.

27) *Moringa oleifera* Linn. (Local Name: Shevaga, Family-Moringaceae).

**Edible parts**-Fresh leaves, tender pods, and even flowers are edible.

**Tribal recipe**-young leaves and flowers are steamed before being mixed with gram flour, salt, and chilies in a tribal recipe. Only salt and chilies are used in the tribal habitat's inner.

**Ethnomedicinal applications:** This vegetable is used to treat eye issues, indigestion, and snake poisoning.

28) *Nymphaea lotus* Linn. (Local name-Bhishi, Kamal, Family-Nymphaeaceae).

**Edible part**: Underground tubers

**Tribal recipe**-Washing 4-5 times and then cooking with salt is a tribal dish.

**Ethnomedicinal applications:** Rhizome is utilised in the treatment of diarrhoea, dysentery, general debility, and heart problems. In indigestion, vomiting, and dysentery, root stock churna is employed. A decoction of tubers is provided in the case of excessive heat in the body, as well as during the passage of seminal fluid through the urine.

29) *Paracalyx scariosus* Roxb. (Local Name-Ran Ghevda, Family-Fabaceae).

**Edible part**-young leaves and pods

**Tribal recipe-** Fresh young leaves are cooked with onion, salt, and chilies in a tribal recipe. Beans are removed from the pods and consumed after they have been boiled with salt.

**Ethnomedicinal applications:** Ethnomedicinal uses include scorpion bites and leg cramps.

30) *Portulaca oleracea* Linn. (Local name-Ghol, Family-Portulaceae).

**Edible part-** Stem and leaves

**Tribal recipe**-Fresh stems and leaves are lightly cooked and eaten with onions and chilies in the tribal recipe.

**Ethnomedicinal applications:** Seeds are a cooling diuretic that can be used to treat jaundice and diarrhea. They can also be used to treat burns and scalds. Skin problems, abscesses, wounds, coughs, and swellings are all treated with this herb. Also used for tiredness, difficulties breathing, and vision problems.

31) *Praecitrullus fistulous* Stocks (Local Name-Dhemse, Family-Cucurbitaceae).

**Edible part**-Fruit

**Tribal recipe-**Fresh fruits are eaten like a vegetable with salt and chilies in tribal recipes, and they are also utilized in curries.

**Ethnomedicinal applications:** Ethnomedicinal uses include the treatment of fevers, blood infections, and asthma.

32) *Smithia purpurea* (Local Name-Kaula), Family-Papilionaceae

**Edible part**-Leaves

**Tribal recipe**-Chopped leaves are cooked for a few minutes on a low flame in this tribal recipe. A sufficient amount of oil, salt, and chili is used. When combined with crab legs, it is supposed to form a delicious dish.

**Ethnomedicinal applications:** Anthelmintic properties are found in the juice of the leaves. Malnourished babies are fed a paste made from young leaves to help them gain strength.

33) *Solanum verbascifolium* Linn. (Local Name Jungli Wangi, Family-Solanaceae).

**Edible part-**young fruits

**Tribal recipe**-Fruits are usually cooked for a few minutes in tribal dishes. Boiled water is discarded, and curries are made with vegetables.

**Ethnomedicinal applications**—In tribal medicine, leaves and fruits are used. Fruits have a laxative effect. Toothaches are treated with seeds.

34) *Spondius pinnata* (Linn.) Kurz. (Local Name-Ambada, Family-Anacardiaceae)

**Edible part**-Fruit

**Tribal recipe:** The fruits are sliced and the seeds removed, then cut into pieces and cooked as a dry vegetable with salt and chilies in a small amount of oil. Fruits are also cooked in curries.

**Ethnomedicinal applications:** The seeds are powdered and applied to rashes, eruptive acne.

Many people in rural areas around the world depend on wild edible plants for food., made several attempts to compile a list of the wild foods found in Maharashtra's Vidarbha region [21-24]. Vegetables are high in vitamin A, and the vitamin B complex, as well as dietary fibre and phytochemicals. The nutrients found in wild vegetables also safeguard our bodies from starvation and nutrient deficiencies. That's why they're referred to as "protecting foods" [25]. These unusual wild edible plants are rich in micronutrients and trace elements, as well as proteins and lipids [26,27]. According to a review of the research, eating enough vegetables can prevent you from chronic diseases like cancer, obesity, diabetes, cardiovascular disease, and metabolic syndrome.

## 4. Conclusions

People will have to depend on wild edible plant resources as an alternative to conventional ones in the near future to meet the increasing demands for food and nutritional requirements due to the increasing population pressure. As a result, plant resources play an important role in resolving a variety of issues such as shelter, food, and medicine. As a result, many of these wild plants have a high cultural value among the locals and are thus linked to their indigenous traditions.

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ARTICLE
# Volatile Constituents of Leaves of *Trifolium alexandrinum*

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ABSTRACT

This study presents the volatile constituents from leaves of *Trifolium alexandrinum* from Pakistan. The dried leaves were subjected to hydro-distillation for extraction and extracted volatile oil was subsequently characterized by gas chromatography-mass spectrometry technique. A total of 22 compounds were identified where the most dominant constituents were: phytol (46.00%), palmitic acid (9.26%), phytol acetate (6.44%), and linolenic acid (3.47%). Most of these are well-known constituents to exhibit antibacterial, antioxidant, antitumor, and anti-inflammatory activities.

## 1. Introduction

In many developing countries (Pakistan, India, Bangladesh, Nepal, etc.) a vast majority living in rural areas not only utilize but also rely on traditional and herbal sources rather than synthetic medicines as they cannot afford expenses of the latter [1]. Additionally, natural remedies are preferred [2] and essential oils of aromatic plants have been employed as a useful source against various ailments because of their vast and diverse bioactive properties [3]. Because of fertile lands, these countries have diversity in plants of medicinal importance, and a variety of medicinal plants are grown and harvested. Although, many plants have been studied, however, there are numerous other plants either uninvestigated or have rare literature on them, and hence, there is a great need to explore their essential oil composition and medicinal properties especially antimicrobial activity [4-6].

*Trifolium alexandrinum* is an important winter fodder crop in Egypt that has been cultivated since ancient times [7]. It belongs to a family Fabaceae, commonly called berseem clover or Egyptian clover [8] that possesses

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antibacterial [9], hepatoprotective [10], phytoremediation [11], and antidiabetic [12,13] activities. It is distributed in Pakistan, Egypt, Syria, Iran, South Africa, South America, Italy, India, and Australia [7]. Most species of this genus have been utilized as folk medicine in many countries. In Turkish traditional medicine, they are used as analgesics, antiseptics, and expectorants [9]. The flowers of Egyptian clover are yellowish-white in color, stems are hollow with alternate leaves possessing oblong leaflets. It grows upright as tall as 18-30 inches and contains 18-28% crude protein [8]. Berseem clover has become one of the fastest spreading fodder species of recent time. According to the most recent findings of an ethnobotanical survey conducted in Pakistan, the seeds of *T. alexandrinum* are a source of antidiabetic treatments, and the dried flowers are used to cure asthma, congestion, and ulcer, among other ailments. Wound healing, on the other hand, is accomplished using the entire plant. It is standard procedure in Iraqi Kurdistan to make decoctions from this clover leave for the cure of diarrhea [14].

Berseem may only be grown by seed, which is often sowed in the early fall months. It can be planted in a normal breeding ground or directly injected into the ground. Seeds of berseem can be planted alone or in a mixture with other plant species. It is combined with grasses (ryegrass) or with a seasonal grain crop such as oats to yield sufficient silage that can be stored for several months. It can be incorporated into rice-wheat crop production as a winter and early spring nutrition crop, and it is planted before or immediately after rice production to provide nutrients to the rice yield. In Australia, it is cultivated with other pulses such as balansa clover (*Trifolium resupinatum*), arrow leaf clover (*Trifolium vesiculosum*), and Persian clover (*Trifolium resupinatum*) to produce a more diverse crop. It is sometimes mixed with vegetables such as turnips or sarson (*Brassica juncea*) in various regions [15].

Legume family is one of the largest plant families, and the clover belongs to the genus Trifolium of this family [16]. Approximately 240 different clovers species [17] are found in the *Trifolium*, which can be found in subtropical and temperate locations throughout both continents. It has been discovered that mountains of east Africa, Mediterranean basin, and northwestern America are all home to *Trifolium* species, whereas there are no *Trifolium* species found in the southeastern Asian and Australian continents [16]. Several plants of this genus are being used as herbage plants for generations (e.g., *T. pannonicum Jacq., T. repens* L., *T. medium* L. etc.) apart from their use as traditional medicine in diverse cultures [16].

Clovers are extensively used for the treatment of psoriasis and eczema for thousands of years, both in Asian and Europeans countries alike. As cough suppressant, antiseptic, painkiller, relaxant, and stimulant mixes*, T. repens* L.*, T. pratense* L.*,* and *T. arvense* L. have been used in ancient Turkish medicine [17]. In the Mediterranean region, few species of this genus are grown as fodder for animals [9]. *T. repens L. and T. pratense L.* are prominent medicinal herbs used in Pakistan, and are effective for the treatment of pneumonia, sinus infections, fevers, encephalitis, and a febrile sensation in the body [18]. Native Americans have traditionally employed these medicinal herbs to treat exterior skin issues, lung ailments, as well as various abnormalities of the mental and hormonal systems [8]. *T. pannonicum Jacq.* is a medicinal plant that grows wild, and is used by the locals for recovery of injury during the process of healing [19]. It is one of the most common natural pharmaceutical plants in the region. It was investigated that *T. angustifolium* L. extracts are used as the herbal remedy in Portugal to treat stomach cramps and diarrhea [20]. Egyptians have used the seeds of *T. alexandrinum L.* as an anti-diabetic medicine for thousands of years. *T. repens* L. is an antiparasitic cure used in herbal medicine by the Nagaland tribal communities to treat intestinal parasites. An in vivo investigation on animals has revealed that white clover has anti-inflammatory properties [16].

Over the last three years, more than 20 modern research works have been reported verifying the ethno - medicinal effectiveness of different *Trifolium* species. However, red clover is considered to be well-known among the *Trifolium* species, and it has been extensively studied in terms of therapeutic potential (particularly estrogenic effects) and agronomic relevance [14]. Red clover extracts are available for purchase as nutritional supplements in the marketplace. Although the antibacterial activity of isoflavones may have therapeutic efficacy for treating the disorders associated with hormones imbalancement, such as heart disease, menopausal problems, cancer, and osteoporosis, but there is currently no evidence to support this claim in the scientific community [21].

The production of phenolic and polyphenolic chemicals by *Trifolium* plants, in addition to isoflavones, is well documented. These compounds include phenolic acids, flavonoids, clovamides (caffeic acid esters), saponins, and a variety of other compounds. The apical parts of 57 *Trifolium* plants have been examined for their clovamide content, flavonoids (mostly isoflavones), phenolic acids, and then divided in 5 groups based on the results of the analysis [16]. There are several species in Cluster 1 that have the highest percentages of isoflavones (51-97 mg/g of dry mass), including *T. medium* L.*, T. lappaceum* L.*, T. phleoides* Willd. etc. where *T. bocconei* Savi. and *T. angustifolium* L. are the members of Cluster 2, that contains species having very high flavonoid content of about 16- 32 mg /g of dry mass. Among plants of Cluster 3 are *T. isthmocarpum* Brot*., T. resupinatum* var. *majus* Boiss*.,* etc. with low total phenolics. The species in cluster 4 have a high content of phenolic acids, ranging from 1-1.8 percent of dry matter [16]. Plants belong to Cluster 5 contain high levels of phenolic content. Each of these clovers exhibited substantial quantities of clovamides, flavanoids and polyphenolic compounds [16].

However, chemical studies on different parts of *Trifolium alexandrinum* showed the existence of proteins, isoflavonoids, flavonoids and their glycosides, steroids, terpenoids, amino acids and their derivatives, and fatty acids [8,9]. Considering the significant pharmacological properties and very rare information on its volatile composition, its aroma was studied.

## 2. Materials and Methods

### 2.1 Plant Material

Leaves of *Trifolium alexandrinum* were purchased from Akbari Market, Lahore, Pakistan. Drying, cleaning, and grinding of plant samples were carried out till a fine powder was obtained. 100 grams of the powdered sample was subjected to hydrodistillation for 5 hours and n-hexane was used as the collecting solvent through solvent extraction technique. The organic layer containing the volatile oil was then separated. The sample was stored in an air tight sample vial containing septum for the desired purpose and then kept at a low temperature (−10 ℃) for GC-MS analysis.

### 2.2 Gas Chromatography-Mass Spectrometry

Agilent 5977A series GC-MSD system was used to perform separation and characterization of volatile oil. A nonpolar capillary column, DB-5 MS (30 m × 0.25 mm ID × 0.25 μm film thickness) was used for separation of volatile compounds where operating conditions were: starting temperature was set at 100 °C and it was raised to 310 °C at a rate of 15 °C / min and then held for 2 mins. The flow rate of helium was 1 mL/min. An injection volume of 1 μL in split mode (split ratio 15:1) was used with a total runtime of 16 minutes. The conditions for the mass detector were: source, transfer line and MS Quad temperatures were 230 °C, 280 °C and 150 °C, respectively, where *m/z* was set in the range of 37-500.

## 3. Results and Discussion

The light yellow oil was obtained and the ion-chromatogram for separation is shown in Figure 1. A total of 22 compounds were successfully identified that included numerous bioactive compounds and are provided in table 1 where most of the identified compounds showed matching quality above 90%. Major constituents identified were: phytol (46.00%), palmitic acid (9.26%), phytol acetate (6.44%), and linolenic acid (3.47%). Many of these are known to be aromatic and flavor imparting compounds such as phytol possesses a floral type odor, palmitic acid exhibits a faint oily fragrance and phytol acetate has a waxy odor.

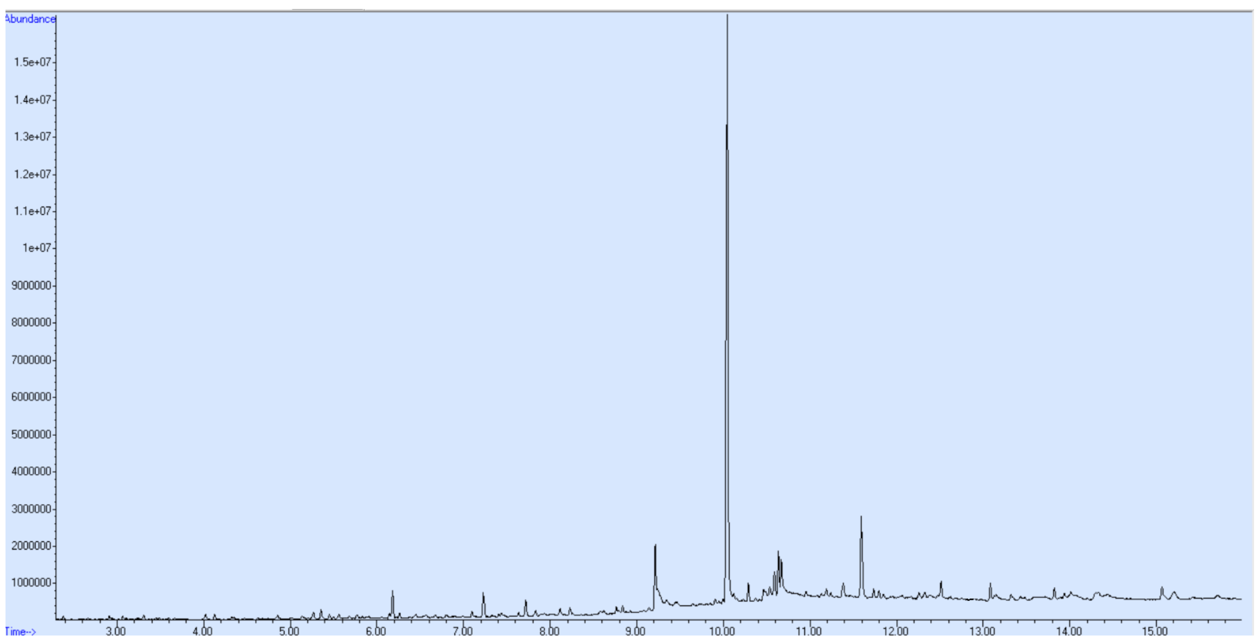


**Figure 1.** Total Ion Chromatogram of the volatile oil of leaves of *Trifolium alexandrinum*

**Table 1.** Volatile composition of leaves of *Trifolium alexandrinum*

| Sr. No. | Retention Time (min) | Identified Compounds | *Abundance (%)* | *Retention Indices ($RI_{Lit}$)* |
|---|---|---|---|---|
| 1. | 2.470 | p-Cymene | T | 1026 |
| 2. | 3.162 | Durene | 0.11 | 1115 |
| 3. | 4.021 | Safranal | 0.55 | 1173 |
| 4. | 4.123 | β-Cyclocitral | 0.45 | 1196 |
| 5. | 4.318 | β-homocyclocitral | 0.23 | 1235 |
| 6. | 5.35 | β-Damascenone | 0.79 | 1354 |
| 7. | 5.56 | Damascone | 0.42 | 1383 |
| 8. | 6.18 | trans-*β*-Ionone | 2.02 | 1470 |
| 9. | 6.26 | β-Ionone epoxide | 0.36 | 1488 |
| 10. | 7.83 | Ar-tumerone | 0.52 | 1664 |
| 11. | 8.23 | Hexahydrofarnesyl acetone | 0.67 | 1833 |
| 12. | 8.76 | Isophytol | 0.43 | 1920 |
| 13. | 8.83 | Methyl palmitate | 0.43 | 1930 |
| 14. | 9.22 | Palmitic acid | 9.26 | 1960 |
| 15. | 10.04 | Phytol | 46.00 | 2105 |
| 16. | 10.28 | Linolenic acid, methyl ester | 1.21 | 2125 |
| 17. | 10.46 | Oleic acid | 1.52 | 2141 |
| 18. | 10.54 | Linoleic acid | 1.10 | 2145 |
| 19. | 10.58 | n-Nonadecanol-1 | 1.66 | 2156 |
| 20. | 10.67 | Linolenic acid | 3.47 | 2143 |
| 21. | 11.59 | Phytol acetate | 6.44 | 2218 |
| 22. | 11.79 | Tricosane | 0.42 | 2300 |

T = trace > 0.1; $RI_{Lit\,=}$ Retention indices from literature

The spectra of major compounds are provided in Figure 2 and their fragmentation patterns and relative spectral data is provided as under:

Phytol: 297(<1, M+), 278(1), 123(28), 111(9), 95(16), 83(16), 71(100), 57(29). Peak match was 98%.

Palmitic acid: 258(1, M+), 257(4), 157(13), 129(41), 115(16), 73(100), 60(80), 57(71), 55(74). Peak match was 99% 71(100),

Phytol, acetate: 296(2, M+), 278(5), 151(4), 137(9), 123(58), 109(21), 95(50), 82(46), 68(64), 55(46). Peak match was 76%;

Linolenic acid: 204(1, M+), 189(1), 175(2), 161(1), 147(3), 133(4), 128(2), 121(16), 105(11), 93(49), 79(100), 67(79), 55(87); Peak match was 99%.

Numerous well-known and significantly bioactive constituents such as cymene, safranal, linolenic acid, linoleic acid, phytol, and palmitic acid were obtained from these oils. Phytol is a diterpene alcohol [22] and palmitic acid belongs to fatty acid class of compounds. Phytol being the major constituents of this oil where numerous studies have reported its pharmacological activities such as antibacterial [23], antioxidant, antinociceptive activities [24], antiallergic, anti-inflammatory [25,26], antiquorum [27], cytotoxic [28] and antitumor activities [29]. Besides, it has immunostimulant [30] and anticonvulsant properties as well [31]. It also acts as a precursor for the formation of vitamin E and $K_1$ [32]. Phytol acetate is a diterpenoid derivative of phytol and have been reported for its remarkable antifungal, antibacterial [33] anti-diuretic and anti-inflammatory [34] activities. On the other hand, palmitic acid is a well-known fatty acid that possesses antitumor [35], and anti-inflammatory activities and medicated oils rich in palmitic acid are used to treat rheumatic symptoms in the traditional medicine of India [36]. On the other hand, linoleic and linolenic acids are dietary essentials that also possess various medicinal properties. Linolenic acid is an omega-3 fatty acid whereas linoleic acid is an omega-6 fatty acid [37]. Linolenic acid, methyl ester has antioxidant and antimicrobial properties [38]. β-damascenone and damascenone are rose ketones and the former has been reported for it antispasmodic and anti-inflammatory properties [39]. Safranal is minor constituent of plant essential oil and it is reported for its antifungal [40] properties. Safranal also exhibits

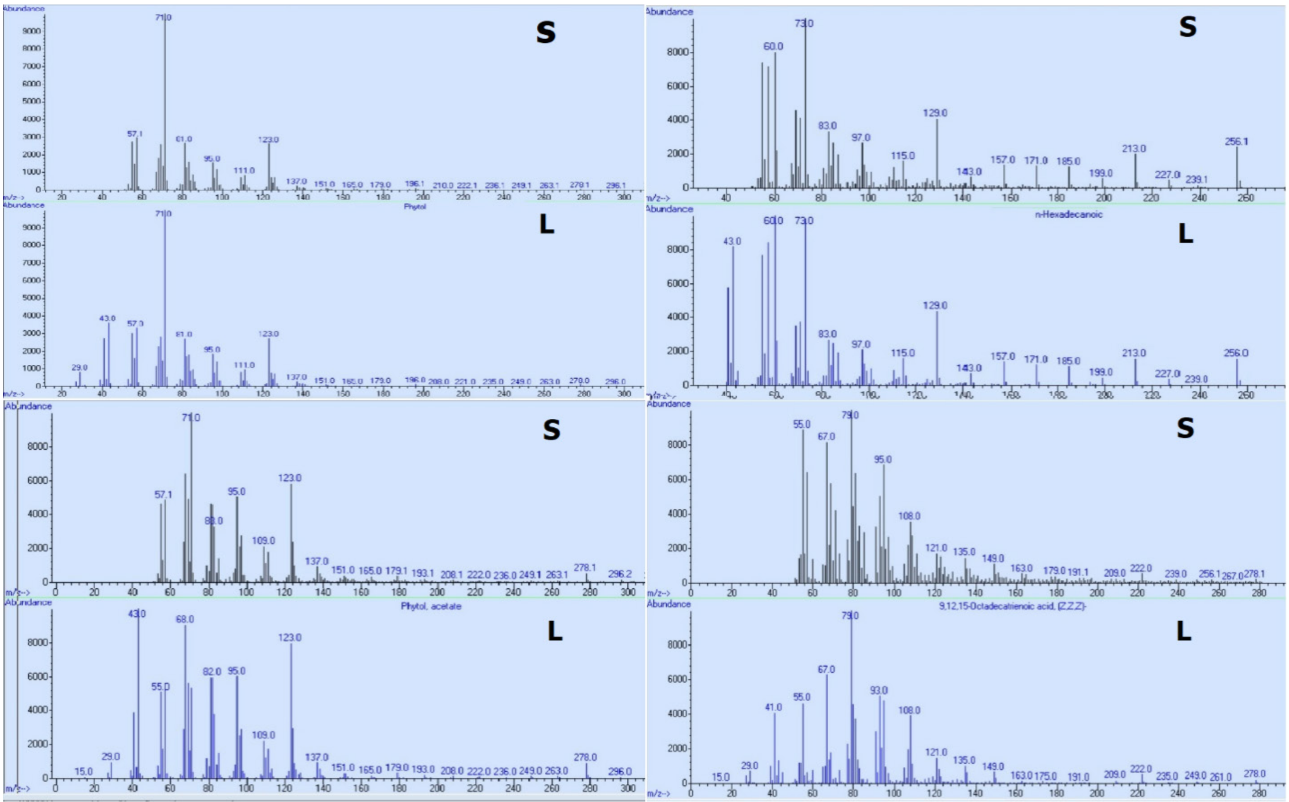


**Figure 2.** Matching mass spectra of major compounds.

S = sample spectra, L = Library match

anticonvulsant and antioxidant bioactivities. β-Ionone epoxide is a monoterpenoids derivative of β-Ionone which is a yellowish liquid. β-Ionone and its derivatives act like anticancer agents [41]. Immunological assay of ar-tumerone which is sesquiterpenoid that shows its strong potency against snake venom and thus used as a medicine for the treatment of snake bite [42]. p-Cymene is an important pharmaceutical component which is used to treat coughs and phlegm and is a monterpene having antioxidant properties which is used to cure ailments in which oxidative trauma shows pathophysiological role [43]. *β-Cyclocitral* is a monoterpene which possesses notable antimicrobial and antioxidant characteristics [44]. Besides, numerous other minor constituents are also present that exhibit aromatic and bioactive properties. The classification of the identified constituents is provided in Table 2. The major classes belong to terpenoids (including terpenes and terpenoid alcohols) and fatty acids where these classes also known to exhibit various pharmacological and aroma imparting properties [45-47].

**Table 2.** Classification of volatile oil constituents

| Sr. No. | Class of volatile constituents | Serial numbers of compounds from Table 1 | Percentage (%) |
|---|---|---|---|
| 1. | Terpenes & Terpenoids | 1, 4, 8, 9, 21 | 9.27 |
| 2. | Fatty acids | 14, 17, 18, 20 | 15.35 |
| 3. | Ketones | 6,7, 10, 11 | 2.4 |
| 4. | Terpenoid alcohols | 12, 15 | 46.43 |
| 5. | Aldehyde | 3, 5 | 0.78 |
| 6. | Esters | 13,16 | 1.64 |
| 7. | Others | 22, 2, 19 | 2.19 |

## 4. Conclusions

The volatile oil extracted from the leaves of *Trifolium alexandrinum* was found to contain a total of 22 constituents. In this study, it was discovered that phytol, a pharmacologically significant constituent, was present as one of the major constituents, and that it is associated with a variety of bioactive properties. There were several major and minor active constituents present, which led us to propose that further bioactive studies should be conducted in order to evaluate the bioactive potential of this volatile oil.

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REVIEW

# Current Standing of Longleaf Pine Trees under Climate Change

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ABSTRACT

Climate change poses many risks to economically and ecologically crucial species. Longleaf pine (*Pinus palustris* Mill.) trees are keystone species that were once dominant across the southeastern United States, but now occupy less than 5% of their historic range and are thus classified as endangered. Here we review the current status and challenges facing longleaf pine trees, what is known on how changing climate will impact longleaf growth and reproduction, and gaps in the literature that are important to address. We found that many fundamental aspects of longleaf pine growth and reproduction are understood. However, these systems are complex, and not all is known about each factor that influences the relationship between climate, growth, and reproductive output. Additionally, long-term data sets capable of examining all relevant factors in these relationships do not currently exist. To fill necessary gaps, we recommend a joint approach between using readily available data sets and establishing new long-term monitoring plots targeted to collect data on missing or poorly understood conditions. This review provides a clue from an ecological complexity perspective to understand and manage longleaf pine forests under climate change.

## 1. Introduction

Climate plays a crucial role in biodiversity and plant community structure, and anthropogenic climate change is expected to pose a significant threat to many ecosystems [1,2]. Moreover, the climate is a primary factor in constraining species range, and global climate change has already been shown to impact the dispersion of species [3]. Relevant climate variables on plant success can include annual average temperature or precipitation, a total number of days above or below a certain temperature threshold, frequency of high-intensity events such as hurricanes or droughts, and many others [4-6]. Moreover, changing climate can affect plant communities on both short-term and long-term scales [1]. However, each taxon responds differently to changes in each variable, and much is still not understood about how a globally changing climate will affect every plant species [1,7-9]. More genetically or spatially malleable species may adapt

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or experience range shifts, while more constrained species may face extinction [7,10].Vincent et al. [11] supported that rarer (i.e., less widespread) wild plant species were less resilient to changing temperatures and precipitation than their more common counterparts, possibly due to their inherently more constrained niche.

In the southeast United States, climatologists anticipate a continued increase in temperatures and higher variability in precipitation, with a greater occurrence of extreme precipitation events such as hurricanes [12]. Regional variability is expected as well, and confounding factors in many locations will result in increased drought stress [4,13]. This has many implications for forest ecosystems since climate impacts the dynamics of both trees and stand success, although how can vary drastically by species [14-16]. Climate change thus poses many new questions of how endangered plant species will transform over time.

Southeastern pine trees have been massive sources of profit for hundreds of years, and once produced the greatest amount of rosin and turpentine ever harvested globally [17]. Even still, southeastern US pine plantations produce 16% of the world's industrial wood supply [18]. This does not include the economic benefits of practices such as agroforestry or nontimber products, which can significantly enhance the value of land[19-20]. Current sources of profit are largely from pine plantations of more frequently selected for species such as shortleaf pine (*Pinus echinata* Mill.), slash pine (*Pinus elliottii* Engelm. var. *elliottii*), and loblolly pine (*Pinus taeda* L.). However, one less widespread species, longleaf pine (*pinus palustris* Mill.), has been the subject of growing interest and concerted restoration efforts over the last few decades [21,22].

In the time before European settlement, longleaf pine savannas covered 60-90 million acres or more across the southeastern United States, running north-south from Virginia to Florida and as far west as Texas [17,21] (Figure 1). An estimated 62% of this area was dominated singularly by longleaf pine trees [17]. However, following colonization, southeastern pine trees were widely depleted due to over-harvesting of naval stores and timber. Increasing land-use change, along with other factors prevented the reestablishment of much of previously forested areas [17]. Even more so than its counterparts, longleaf pine experienced lower success in reestablishment due primarily to suppressed fire regimes and young tree predation from nonnative feral hogs [17]. This, along with other factors such as sporadic seed crop production and selection for other pine species for use in pine plantations, has resulted in a current longleaf pine coverage of less than 5% of its historical range, and both the tree and ecosystem are classified as endangered [17,21].

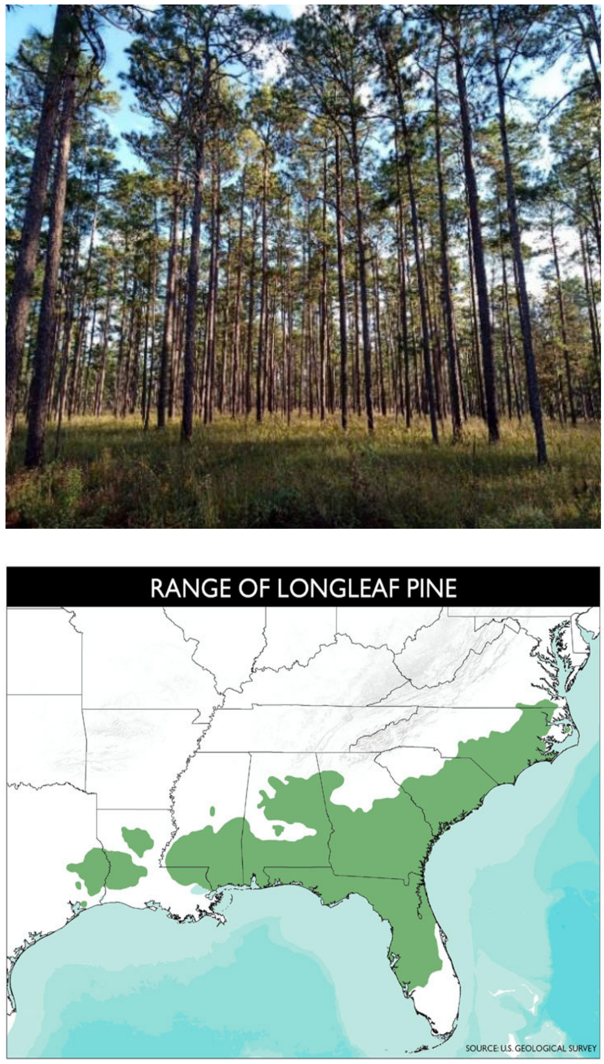


**Figure 1**. Longleaf pine forests and the pre-settlement range.

Despite the high fragmentation and poor quality of remaining stands, longleaf forests still hold much ecological and commercial value [21,23]. Commercially, longleaf pine trees produce a high-quality wood that is likely more resilient to damage due to natural stressors such as drought [18,24,25]. Longleaf stands are also ideal for alternative sources of revenue, such as pine straw and wildlife leases [17]. Ecologically, longleaf trees remain keystone and indicator species when dominant in the canopy [21,26]. Remaining longleaf ecosystems host some of the highest levels of biodiversity in North America, and are home to many endemic species that now face declines due to habitat loss [17,21,23]. Perhaps most highlighted of these is the federally endangered red-cockaded woodpecker (*Picoides borealis* Vieillot), which acts as a keystone species due to its unique ability to create cavities in still living trees[27]. Environmentally, pine forests act as carbon sinks, even more so than hardwood forests, and even when managed through frequent burning [28]. It has even been suggested that longleaf pine ecosystems have the greatest potential for carbon storage of southeastern pines given their life history, commercial use, and ecological characteristics [29].

Most remaining longleaf ecosystems are altered beyond their resiliency and require human intervention to survive [17]. In the latest US Forest Service report on longleaf pine forest conditions, Oswalt et al. [21] report that the primary cause of mortality in longleaf pines is damage from weather events (eg. windthrow, lightning). However, altered fire regimes, high levels of fragmentation and disturbance from invasive species, as well as limited amounts of harvesting for timber may also impact stand success [21]. However, much is still not understood about how climate change will impact each of these factors, as well as the trees themselves. If climate change can affect environmental, conservational, and economical sectors of forest ecology, and longleaf pine is a crucial southeastern species, then more must be known about how changing climate will impact longleaf pine success [30-32]. Here we outline what is known and highlight existing gaps in our current understanding of how climate change is expected to impact longleaf pine growth and reproduction, two key metrics for conservation.

## 2. Climate and Longleaf Pine Growth

Longleaf pine grows from April through October in its northernmost areas of range to almost year-round nearer to the equator, where growth intermittently stagnates in the months of January through February [33,34]. It is characterized by slower early-stage growth than many other cooccurring pine species, due to its formation of a grass stage that is not shared with other southern pines [17,22]. This stage acts as a trade-off, however, as it significantly increases the ability of individuals to survive low-intensity fires that otherwise kill off competitor trees [21,35]. Without the competition of fire, longleaf pine will often be outgrown and outcompeted by other tree species [17].

Much about the relationship between climate and growth of longleaf trees comes from correlative studies using dendrochronological records [34,36,37]. Dendrochronology is a well-purported dating method capable of determining growth, climate, disturbance, and anthropogenic histories from living trees and preserved wood [38,39]. Advances in dendroclimatology allow for higher resolution in determining spatial and temporal patterns from tree ring data [9]. However, growth responses to climate variables differ widely between species and potentially over time [9,33,40]. Although many datasets only consider total annual tree growth, total wood (TW) is comprised of two parts: earlywood (EW, spring growth) and latewood (LW, summer and fall growth) [6,34]. Species-specific EW and LW growth each often correlate with seasonal climate variables and therefore allow for a more complete climate history [6,41]. However, difficulties in distinguishing seasonal growth factors can arise given the confounding relationship between interannual EW and LW growth, which is also known to differ between species. This variation, along with other physiological factors, can determine how useful a species is in establishing chronologies [34,41].

Longleaf pine is a quality dendroclimatological species given its historical abundance, slow decay, climactic sensitivity, and long lifespan [33]. Multiple centuries-long chronologies from longleaf growth rings have been produced [34,36,37]. Another useful characteristic of longleaf pine is that the transition from EW to LW is characteristically discrete and occurs relatively consistently between May and June [33,34]. In longleaf pine rings, EW is much less sensitive to climate than LW; studies have found weak to no correlation between EW growth and climate variables [34,37]. Interestingly, Stambaugh et al. [34] reported that EW growth was correlated to the previous year's LW growth as suspected, but that changes in sign occurred over time. However, EW growth does vary annually, and there are irregular strong to insignificant positive correlations between EW and LW growth in the same year [34,37]. Thus, Soulé et al. [37] found that the best chronologies are obtained from LW growth that is adjusted to remove the influence of EW trends.

Multiple studies have reported on the strength of late summer and early fall climate correlations to longleaf radial growth [34,37]. Soulé et al. [37] found a positive correlation between growth and mean temperature during the first half of the year (weaker) that switched to negative around June (stronger) when the transition to latewood occurs. Multiple studies have found positive and negative growth correlations to annual precipitation and temperature, respectively [34,37,42]. In studies that partitioned TW into EW and LW, these correlations were strongest in the late summer/early fall [34,37] (Figure 2). Furthermore, the frequency of high-intensity precipitation events such as hurricanes are positively correlated with LW growth, and can even produce stronger correlations than a greater total amount of precipitation from lower-intensity events [6,37].

Significant gaps still exist in our understanding of climate-related variation in longleaf pine radial growth. For example, Ames et al. [42] inexplicably found that the individually detrimental effects of fire and

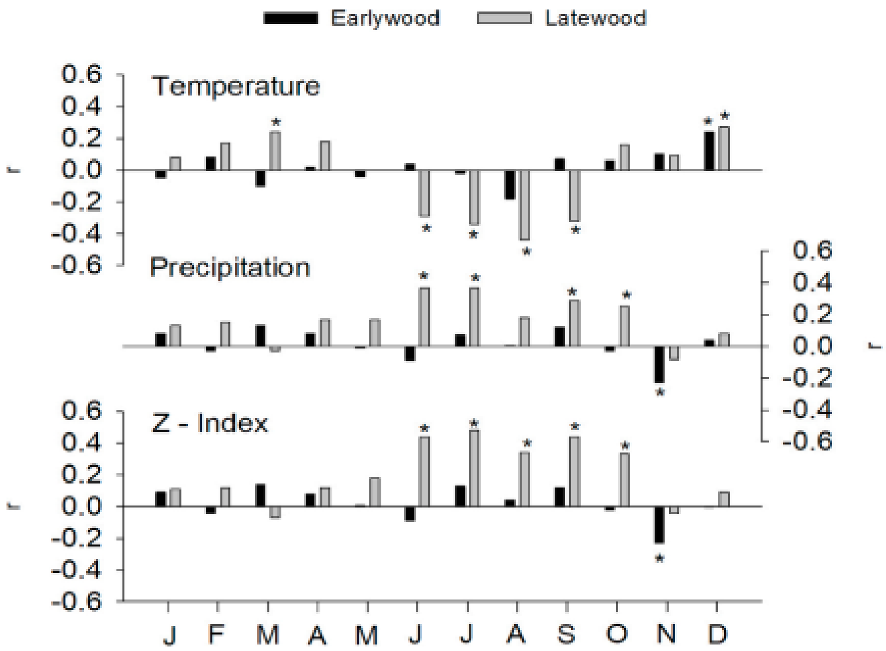


**Figure 2.** Earlywood and latewood correlation coefficients for monthly temperature, precipitation, and Palmer Z-Index from longleaf pine wood. Earlywood and latewood tree-ring chronologies. Stars indicate statistical significance at the α=0.05 level. From Stambaugh et al., 2021.

high temperatures on growth can mitigate each other. Additionally, a growing number of studies have begun to challenge basic assumptions in our understanding of longleaf growth. Loudermilk et al. [43] found that, in opposition to conventional knowledge, the presence of midstory oak trees on xeric sites can facilitate the survival of longleaf pine seedlings, likely due in part to the reduction of low soil moisture stressors. However, given competition of other resources, more must be studied on how early growth rates (e.g., biomass accumulation) or growth of longleaf pines on wetter sites may be impacted. Furthermore, the ease of climate manipulation for younger trees along with a focus on longleaf reestablishment in research has led to an overabundance of experimental studies on seedlings and saplings, and fewer studies exist that manipulate climate variables for whole stands of mature trees [44-46]. When experimental manipulation is conducted on mature stands, methodology is limited to what is spatially and environmentally possible; Samuelson et al. [12], for example, lowered soil water content by throughfall exclusion troughs but were still constrained by naturally occurring drought conditions. Moreover, given the known variation in response to climate across longleaf pine range, as well as the limited geographical extent of most chronologies, more analyses are needed to evaluate if each of these patterns is universally true rather than site-specific.

## 3. Climate and Longleaf Pine Reproduction

Although fundamentals of longleaf pine reproduction are generally understood, many factors related to climate variability are not [47]. Longleaf pines are wind-pollinated and monoecious [35]. The reproductive cycle lasts approximately 3 years from the time reproductive strobili begin to form until seed dispersion occurs (Figure 3) [48]. Seed production is temporally variable; masting cycles have been contradictorily reported over average time frames from 3-10 years, and are now understood to vary spatially [47-49]. Physiologically, the resource accumulation hypothesis suggests that masting species, characterized by periods of low seed productivity followed by periods of booms, spend multiple years accumulating resources until a threshold is reached, allowing for large seed production [50]. Chen et al. [50] found evidence for weak phase coupling, yet this varied between sites and only occurred during certain time periods. Thus, longleaf pine is not a strong masting species. Recent work by Chen et al. [51] presented burstiness - strong intermittent activity between longer periods of lulls - of longleaf cone production as a new method for potentially helping to compare and predict the timing of good seed crop years between longleaf pine stands. Many factors such as genetics and site characteristics have been studied and shown to impact these reproductive trends, but relationships with climate are often complex and not fully understood [47,52,53].

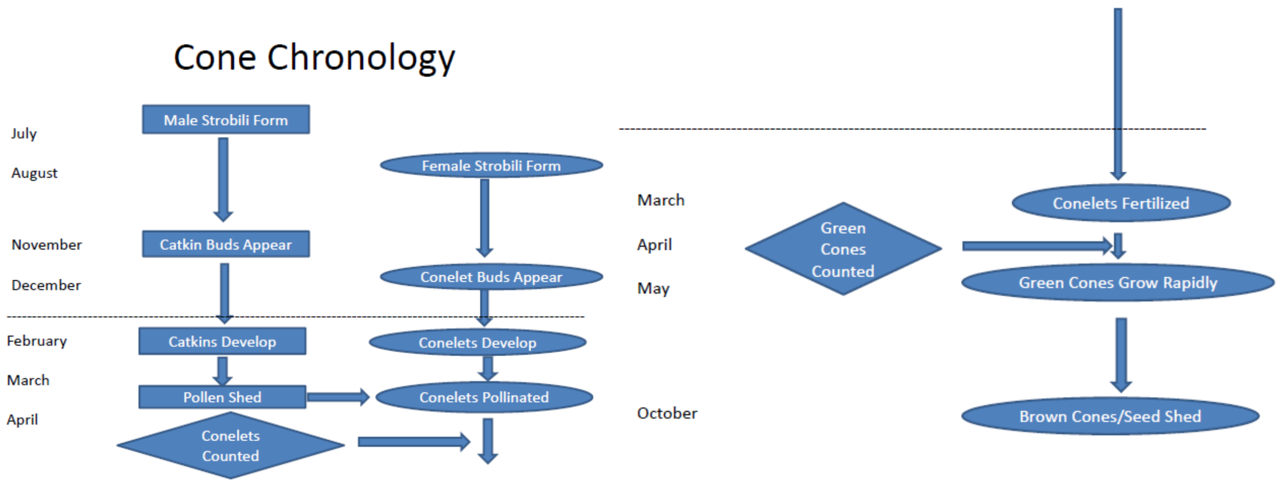


**Figure 3.** The cone chronology of longleaf pine.

### 3.1 Pre-fertilization Trends

As climate changes over time, reproductive phenology, a branch of biology focused on the timing of yearly reproductive cycles, will become increasingly important [54]. In botany, both the timing of growing seasons and reproduction are known to vary with climate [33,55]. Factors associated with reproduction in plants such as time of pollen release, fertilization, and intraspecific interactions are known to vary by species, and the impact of climate on these factors varies as well [1,5,56,57]. For example, in angiosperms, changing temperature variables can lead to altered seasonal timing between plant flowering and pollinator activity and composition [54]. Limited studies have been conducted on longleaf pine phenology as it relates to climate change. Historical research on reproductive influences supports that weather patterns such as precipitation influence early reproductive structure success, and that mismatched favorable conditions between male (catkin) and female (conelet) structures can lead to heavy losses before fertilization [35,49]. Although pollen and unfertilized conelets do not have a temporal cycle, the sex allocation ratio between male and female structures is positively correlated with temperature, and cone production is optimal when this ratio is intermediate [26]. Chen et al. [5] revealed no significant trend in either the time of peak pollen shedding or time of 80% accumulated pollen density over 55 years of data. However, the time of peak pollen shedding was connected to climate factors, primarily the heat sum of total days above 0 °C. Stambaugh et al. [34] and Rother et al. [33] both rely on the importance of seasonality of longleaf growth for dating significant environmental findings. But more information is needed to fully understand how future conditions will affect the timing of peak longleaf growth and reproduction.

### 3.2 Trends in Cone Production

Cone production, the commonly held metric for quantifying longleaf reproductive success, is supported to be both self-organized and self-similar, given that it significantly follows power laws [58,59]. Oftentimes, correlations between climate and cone production are localized rather than universally held [47,48] (Figure 4). Many longleaf sites have seen increased cone production over the past 40 years, but there is not a universally strong link between cone production and climate [48,59]. Chen et al. [47] found no long-term trend in the coefficient of variation (CV) of cone production, although there were fluctuations and several sudden breaks present. They also reported a slight positive correlation of CV with average air temperature and localized correlations with precipitation. When looked at from the perspective of multiscale entropy there is a correlation between the complexity of cone production and the complexity of local climate variables [60]. Guo et al. [48] examined climate variables during the 3-year reproductive cycle of longleaf pines and found that correlations varied by location and grew weaker with increasing distance between sites. Typically, higher cone production was correlated to sites with moderate climates, as well as a warmer July & August and wetter October & November directly preceding seed fall [48]. Despite advancements in research, unexplained variation in cone production still exists between and within sites. Given the lack of data on longleaf pine resource allocation, quantifying internal factors of resource allocation (e.g. biomass allocation) as they relate to climate variables may be a key step to strengthening our understanding of the mechanisms behind sporadic longleaf cone production [5].

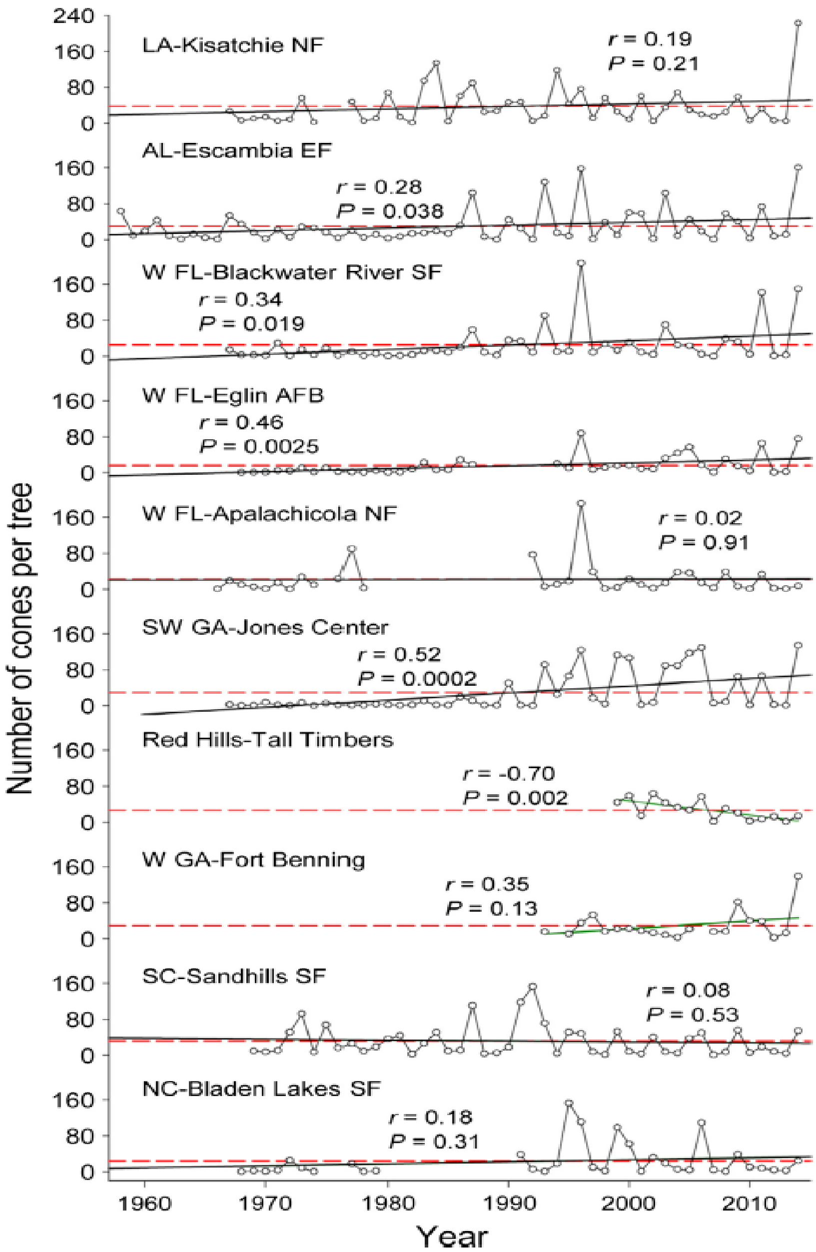


**Figure 4**. Long-term annual cone production at 10 sites across the longleaf pine range using USFS cone data. From reference [48]

## 4. Other Considerations

Wildfire is a substantial factor in longleaf reestablishment and maintenance [17,21,52]. Without the presence of frequent fires, faster-growing yet less fire-resistant hardwoods and other pine species are prone to outgrow and outcompete longleaf pine [17]. Central to this issue is that wildlife regimes throughout the southeast have been anthropogenically drastically reduced since times of pre-European settlement [17,21]. However, relationships between fire, anthropogenic activity, climate, and energy storage can be complex, and not all is known about each factor [52,61]. For instance, the grass stage unique to longleaf pine allows for high survivability of low-intensity fires, yet may represent a trade-off with lowered survivability to drought stress than other pines such as loblolly [35,44]. Despite this, longleaf pine is generally thought to be more drought-resistant than other southern pines given its ability to survive on xeric sites where others cannot, and physiological factors such as a prominent taproot at maturity [44]. Yet it is not known at what exact point saplings transition into developing longer taproots, or if changing environmental conditions drive them to do so at different stages. The knowledge that genetic plasticity has been documented in longleaf pines sourced across its range [62] presents further opportunities for study. Furthermore, more could be known on how management practices of prescribed burning will impact these and carbon storage factors. To understand the role of climate on longleaf conservation, more research is needed on these interactions.

Gaps in knowledge become more confounded when considering the lack of understanding of seed predators, which may play a role in reproductive cycles [63,64]. Although longleaf pine is not a strong masting species, cycles of lesser cone production followed by periods of booms have been noted for decades [17,47,48]. The primary hypothesis for causality of masting in trees, predator starvation and satiation, thus may still apply. This hypothesis holds that the causality of masting is based on extended times of low seed crop reducing predator populations to a point below what can consume the majority of seeds during a boom [63,65]. Recent findings have indicated that the taxonomy of predator species can play a critical role in the effectiveness of masting [66-68]. However, the bulk of studies on masting and seed predation focus on strong masting species [63,66-68]. In longleaf pine trees, the study of the predator starvation and satiation hypothesis is hindered by the lack of a foundational understanding of species distributions and primary drivers of predation pressure. Historical studies have documented fallen longleaf seed predation by insects, small and large mammals, and birds, but many can date back multiple decades [69-72]. Recent localized studies have begun to contradict historical findings in who among these groups are the primary predators (see Willis et al. [73]). Especially considering that wildlife populations can change over time and range, comprehensive datasets capable of connecting masting cycles to predation pressure thus do not exist for longleaf pine. In order to reasonably predict how longleaf pine phenology will impact restoration success, more must be known about how or if seed predators impact reproductive cycles.

It is generally held that, given limited environmental resources, resource allocation to life-history traits such as reproduction and growth should be inversely related as trade-offs [74,75]. However, plant expression of resource allocation can vary over species, location, and time [76-78]. Environmental conditions such as water and nutrient availability are crucial for allocation dynamics, and plants change strategies in response to changing climate conditions [79,80]. When placed under intensive distress from pests or drought, for example, trees may choose to devote most of their remaining resources to either survival or reproduction at the cost of the other [79]. In longleaf pine trees, there is generally a weak negative correlation between cone production and radial growth, but this relationship is largely overshadowed by the influence of other variables such as stand density [81,82]. However, there is often a complex interplay between climate, resource availability, and plant success [83,84], and much must still be understood about reproductive allocation in longleaf pine. For example, frequent, low-intensity burns can hinder growth in the short term [42]. Trees burned more frequently and those higher in more open canopies produce more cones, but when conspecific density is high, larger trees have more reproductive success [42]. Holistic research incorporating data on many relevant factors is needed to understand reproductive allocation in any system, and for longleaf pine, sufficient data sets do not always exist. Moreover, given the many reported findings of inter-site variation in longleaf growth and reproduction [47,48,82], localized approaches will be necessary.

## 5. Challenges and Future Directions

Although short-term studies on the ecophysiological response of longleaf pine seedlings to environmental change exist [17,44], results may be hard to apply for mature trees. We suggest that long-term in-field monitoring at the ecosystem level should be set up in order to better understand the dynamics of tree growth, cone production, and environmental change. This monitoring should include climate, atmospheric conditions (e.g., $CO_2$ concentration and nitrogen deposition rate), soil (e.g., water, nutrients), intrinsic factors (such as photosynthesis), and interactions with other plants (shrubs and grasses). The original pollen observation should be continued. With this long-term monitoring data, the ecological mechanisms related to tree growth and cone production could be discovered. A shortcoming of this research is its likelihood to be cost-prohibitive; for example, photosynthesis (evapotranspiration) of an entire mature tree is hard to measure. Maintaining periods of prescribed burning every three years can also make it complicated for long-term monitoring (e.g., instrument takeoff and re-installation).

Using currently available information and developing modeling or conducting data analysis will be another way to understand the interactions between climate and longleaf pine growth and reproduction. So far, use of US Forest Service cone data has allowed for many studies on influential factors of longleaf reproduction[47,48,50,59,60]. The advantage of this approach is low cost, but the shortcoming is the limited amount of needed information that already directly available. The objectives of the models or data analysis need to be refined by experts in this field.

Thus, a compromise between the above two approaches may be practical, such as setting up important instruments at some study sites for well-developed ideas in long term. In addition, some specific funding opportunities should be available.

## 6. Conclusions

Longleaf pine is a keystone species with significant commercial, ecological, and environmental benefits in the southeastern United States. Yet changing climate poses significant risks to many factors of forest ecology. We present here a limited scope of the current understanding of climate relationships with factors of success of longleaf pine. Growth and reproduction are perhaps two of the most important of these metrics, but not all is known about the fundamental mechanisms behind them. To fully understand and adapt for longleaf pine conservation more holistic and localized research is necessary.

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## Author Contributions

KAB wrote the manuscript and XC provided the ideas and necessary information. Both improved the manuscript.

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