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ROLE OF AGRO-FORESTRY FOR PRODUCTION OF HEALTH AND INDUSTRY BASED ORGANIC COMPOUNDS

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ABSTRACT

Agro forestry is the integration of trees and shrubs into crops and animal farming systems to create environmental, economic, and social benefits for inhabitants any region. *Pinus Roxburghii* (chir pine) *and* similar other species of this family are very important and dominated trees of forest of Murree and Kotli sattian areas (Pakistan). The needles leaves, stem, resins and cones of these trees are important due to their domestic and commercial uses like food, cosmetics and fertilizers. In the current study *Pinus* needles leaves and resins were chemically analyzed to assess levels of phenols, flavonoids, tannins and total oil contents by using different analytical methods as well as GC-MS and FT-IR techniques. Antioxidants, Anti mycobacterium tuberculosis (MT) and cytotoxicity assays were performed to determined bioactivities of different extracts of leaves. Whereas total oil contents of resins were determined by using Soxhlet method and oil was converted into different products like glycerin and biodiesel by using trans etherification process. Results indicates that *pinus* leaves contains total phenols (7.26±1.52) flavonoids (14.53±2.45) and tannins (4.36±1.23). Whereas total oils were found in *pinus* needles leaves (1.92 ± 0.28) as compared to pinus resins. GC-MS analysis exposed higher quantity of organic compounds (fatty acids), those were further confirmed by indication of their functional group by FT-IR analysis. According to results methanolic extracts of Pinus roxburgii has provided higher antioxidant values (DPPH, IC_{50} = 38.36±4.58 µg/ml) and higher zones of inhibition for MT strains as compared to lower value of brine shrimp cytotoxicity assay and these values are probably due to presence of total phenols, flavonoids and essential oils contents. Whereas *pinus* resins results indicates presence of glycerin and fuel oil those have higher economical values especially for developing country like Pakistan

Key words: *Pinus* needles leaves, Resins, Chemical anlaysis, Organic acids, Bioactivities

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INTRODUCTION

Agro forestry is a useful low-cost income applying to many forms of integrated land and to contributes to a green economy by promoting long-term, sustainable, and renewable forest management, especially for small-scale producers (1).

The Pinaceae (pine family) are main tress of forest located on northern east part of Punjab provinces as well as other parts of Pakistan. *Pinus Roxburghii* commonly known as chir pine. A very important tree as its leaves, stem, resins and cone all has commercial uses since ancient time (2).

Pinus belongs to Pinaceae because of having shoot dimorphism, which includes short shoots (fascicles) that have one to eight narrow needles surrounded by bud scales at the base. Strong woody cone scales with the apical structure exposed after the first growing season (bump) and in the mature cone are also typical of the genus Pinus. Currently, Pinus is treated as a monophyletic taxon (3). The subgenus Pinus (pines) has two fibrovascular bundles per needle, diverging pulvini at cataphyll bases, which usually have persistent sheaths. There are two to eight needles per fascicle and the position of the resin canals is polymorphic (septa; internal, medial external); the seed wings are articulated or oppressed (4).

Pinus trees or bushes, including a considerable number of the outstanding conifers of business significance, for example, cedars, firs, hemlocks, larches, pines and spruces. Pine Bark Band: The inward bark can be used as a germ-free band for cuts and scrapes. It is applied to wounds with channel tape, handkerchief, or cordage (3).

Needle leaf tree (coniferous evergreen) have longer, thin leaves that resemble needles. The leaves remain on the tree year-round and are replaced slowly and continuously rather than all at once. The smaller, tighter needles are more waterproof and wind tight than the larger, wider leaves found on broadleaf or deciduous trees (2, 3,4) . Pine needles can be found on evergreen trees throughout the world and can be found on roughly 114 species in the Pinus genus. Most common pines are predominantly from two subgenera of the Pinaceae family, Pinus and Strobus (5).

Pinus needles has been used for animals caring, ceiling of houses and as fertilizers uses since ancient time. Leaves has also been used as feed and food as well as source of

some cosmetic and similar other chemicals (6, 7).

Rosin is a solid resin derived from the oleoresin (crude turpentine) discharged by various species of pine tree. Rosin is extracted by tipping the stem of tree. Natural resins are typically fusible and flammable organic substances that are transparent or translucent and are yellowish to brown in colour (8).

It may also be extracted by heating the oleo-resin to vaporize the essential oils (spirit of turpentine), or through a naphtha solvent process (9). Rosin is used in the manufacture of varnishes, and it is often combined with other resins (8, 9).

Conifer cone is a seed-bearing organ on gymnosperm plants. It is a type of fruit usually wood, avoid to globular including scales and bracts arranged around a central axis. The seeds of cone are delicious and contains important nutrients especially essential oil (10).

Wood of *Pinus Roxburghii* are very important and use domestically as fuel, house hold items and for preparation of furniture etc., Pine needle tea is taken to remove the valuable stuff when one feel influenza like manifestations in body . Essential oils have been widely used since long for bactericidal, virucidal, fungicidal, antiparasitic, insecticidal, medicinal and cosmetic applications. Nowadays they are widely used in pharmaceutical, sanitary, cosmetic, agricultural and food industries (10, 11).

Around 80% of world population depends on home grown solutions for treatment of different human afflictions. Therapeutic plants have the fundamental part in allopathic medications, extensive number of current medications, for example, dioxin, morphine, codeine, ibuprofen, vinblastine, cocaine, emetine, ephedrine, vinocristine, pilocarpine and so forth., have been disengaged from plants (12). After greater progression of manufactured science during the later half of twentieth century over common items, again the enthusiasm of pharmaceutical industry in restorative plants stir and they took a gander at wellspring of natural plants as synthetic platforms for amalgamation of medications. The normal compounds from therapeutic plants are more secure and could be found to supplant the engineered drugs which constitutes around 70% of our medications (13, 14). Numerous medicinal plants contains awesome amount of antioxidant compounds instead of vitamins and carotenoids Therapeutic agents presents in plants possess significance and extraordinary biological activities. Therapeutic herbs displayed more antioxidant activity and contained

fundamentally larger amounts of phenolics and other secondary metabolites than regularly known vegetables and fruits those are considered as rich sources of common dietary antioxidants (15,16)

A basic oil portion from Canella winterana was additionally tried. The antimycobacterial movement of these substances was tried against *Mycobacterium tuberculosis, M. aviumand M. kansasaii* utilizing the Middlebrook 7H11 agar medium, the Bactec 460-TB radiometric system, and assurance of bacterial suitable tallies. Three mixes, to be specific ibogaine, voacangine and texalin, demonstrated antimycobacterium action (17).

The extracts were taken from Urticamembranacea (Urticaceae), Artemesiamonosperma (Asteraceae), and Origanumdayi post (Labiatae). Each of the three plant extracts showed dosage and time-subordinate killing abilities in different human inferred tumor cell lines and essential societies set up from patients' biopsies. The executing action was particular toward tumor cells, as the plant extracts had no impact on essential societies of solid human cells (18, 19)

Various strategies are utilized so far for the extraction of fundamental oil from plant material, for example, steam refining, dissolvable extraction and so forth. The fundamental oil extraction strategy can be isolated into two noteworthy classes considering temperature utilized for extraction i.e extraction at low or high temperature and extraction at room temperature. The strategies are headspace gathering of volatiles, hydro distillation, steam distillation, Soxhlet extraction and water and steam refining (19). The possible role and mode of action of these natural products is discussed about the prevention and treatment of cancer, cardiovascular diseases including atherosclerosis and thrombosis, as well as their bioactivity as antibacterial, antiviral, antioxidants and anti-diabetic agents (20).

MATERIAL AND METHODS

Collection and preparation of samples

The leaves samples of *pinus Roxburghii* (chir pine) and resins were collected in fine plastic bags duly labeled with date and areas of collection of samples. The collection of samples based on ethnobotanical uses by inhabitants of rural areas of Kotli sattian (Rawalpindi) . The resin was extracted from bark of *pinus* tree in fine plastic bags and

treated separately. The samples were identified by a taxonomist by expert from Department of Botany PMAS Arid Agriculture University Rawalpindi and voucher specimen (No. 142) was deposited for future reference. Around 3 kg of plant material were moved to UIBB, PMAS Arid Agriculture University Rawalpindi. for further process (13).

Figure 1. ***Pinus wallichaina***

The samples of *Pinus* needles leaves and *pinus* resins were used for the extraction of various phytochemicals as well as oils and their bioactivities were determined (21).

Plant materials were washed with demineralized water to evacuate undesirable materials including dust. The leaves samples were shade and sun dried followed by oven dried for overnight at 50 °C. The dried samples were ground with electric grinder, sieve 80mesh and saved in plastic bags at lower temperature till further uses. After washing and sun drying, *pinus* resins samples were used for oil extraction (21,22).

Extraction of *pinus* resins from bark of *pinus* tree

Resin is usually collected by causing minor damage to the tree by making a hole far enough into the trunk to puncture the vacuoles, to let sap exit the tree, known as tapping, and then letting the tree repair its damage by filling the wound with resin within few days (22,23). Natural resins are typically fusible and flammable organic substances that are transparent or translucent and are yellowish to brown in colour. The most generalized technique can be the extraction of the drug with alcoholic

solvents and then subsequent precipitation of resin by adding concentrated alcoholic extract to a large proportion of water. The method of distillation or hydro distillation can be used for the separation of volatile oils from resin (23).

Determination of total phenols

The concentration level of phenol in leaves and resins was determined in different solvents and amount was quantified by using method reported by various authors (21). Briefly 100 μl of extract was diluted with 3 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent was added. After 3 min, 2 ml of 20% sodium carbonate was added and the contents were mixed thoroughly. The color was developed, and absorbance was measured at 650 nm in spectrophotometer (Shimadzu UV-1800). Gallic acid was used as standard and different concentrations of Gallic acid were used to draw standard curve ($R^2 = 0.9926$). The amounts of total phenolics were expressed as gallic acid equivalents (GAE) mg/100g of dry matter (19).

Determination of flavonoids

The flavonoid contents of extracts were determined by using method reported by Husain et al. (2008). Briefly an aliquot (1 ml) of extract or a standard solution of quercetin (4mg/ml) was added to 10 ml flask containing 4 ml distilled water and 0.3 ml 5% $NaNO_2$. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added followed by addition of 2 ml of 1M NaOH after 6 minutes and total volume was made up to 10 ml with distilled water. Absorbance was measured at 510 nm with a spectrophotometer and the concentrations of flavonoid in the samples were expressed as mg quercetin equivalent /g of sample (21,22,24).

Estimation of Tannins

The total concentration of tannins in leaves extract was determined by Folin Denis method. It is measurement of blue color formed by the reduction of phosphotungstomolybidic acid by tannins. According to method, the colorometric determination of tannins was carried out by adding 7.5 ml of distilled water to 1.0 ml of extract and standard solution of tannic acid. Then 1ml of sodium carbonate was added following by addition of 0.5 ml of Folin Denis reagent. The solution was diluted with distilled water up to 10 ml and absorbance was measured at 700 nm. The total tannic acid concentration was expressed as mg of tannic acid equivalent per gram of extract (25, 26).

Extraction of oils by Soxhlet method

Oil was extracted from *pinus* needles leaves and *pinus* resins by using Soxhlet apparatus as reported (27). Total 15 grams of a powdered form of a sample was taken in the clean thimble. Then fix the thimble in the loading chamber and connect the loading chamber with the condenser. Added methanol (300 mL) in the flask and connect it to a continuous supply of water. Fix the whole assembly in the heating mental and heat the flask up to 50 ºC. High temperature caused the vaporization of the solvent and vapors moved upward by the tubes and hit the condenser and turned to hot liquid (28).

Conversion of oil into Fuel (Biodiesel) and glycerin

Crude oil contains many impurities such as free fatty acids, phospholipids and sterols whereas refined oil contains the very small amount of free fatty acids and other impurities. These impurities in the form of FFA and water have significant effects on the reaction, glycerides. Alcoholysis is the chemical reaction in which the oil or fat reacted to the methanol followed by alkali (sodium hydroxide) catalyst to produce ester and glycerol. For getting the high yields of ester in transesterification reaction, use of an extra quantity of methanol is preferred (23).

Trans esterification process

In the base catalyzed reaction, the methanol as a solvent and alkali (sodium hydroxide) was used as a catalyst. Different catalyst concentrations of NaOH such as 0.5 %, 1 % or 1.5 % (w/w) may be used along with the methanol. The homogeneous solution of sodium hydroxide and methanol was prepared. Oil has firstly heated to 60 ºC and then cooled to room temperature. Then add the catalyst, and methanol mixture in the oil flask, set the whole in the reaction assembly apparatus, the mixture was stirred at 800 rpm for 2 h. After the complete reaction time, the whole mixture was taken in separating funnel with the passage of time, two layers were separated. The upper layer was methylated ester, and the lower layer was glycerin. The upper layer consists of different impurities such as unreacted methanol, catalyst, water, and glycerin. Furthermore, neutralization and washing process were carried out for refinement of Biodiesel (29).

Physical and chemical analysis of Fuel (Biodiesel)

Different Physical and Chemical analysis were carried out for assessment of quality of Biodiesel like pH, Iodine, acid and saponification values by following AOAC method (28.29).

Analysis of oil for fatty acids with GC-MS

Fatty acid methyl esters: *pinus* needles leaves oil in n-heptane (0.20 g per 2 mL) was transmethylated using a cold solution of KOH (2 mol L−1) (200 μL) and methyl esters (FAME) was analyzed. The composition of extracted oil was examined by utilizing Gas Chromatography-Mass Spectrometry. The hawlett Packard framework was utilized to analyze the sample. The 6890N was outfitted with DB-5 section (30 m length, 0.25mm inner distance across and 0.25 µm stationary stage film thickness) and combined with a HP 5973 Mass spectrometer (MS; Agilent Advances Inc. USA). The injector was worked at 235 °C. The stove temperature of GC was customized as: the underlying temperature of broiler was 40 °C and it was kept up for 2 min, the temperature was raised from 40 to 450 °C at a warming Rate of 4°C and stayed at higher temperature for 8 mins. Exceptionally unadulterated Helium gas was utilized as portable stage with a steady stream of 1mL/min. The weakened arrangement of fundamental oil was infused with volume of 1 µL in split less mode. Mass spectra was performed by an electron ionization framework worked at the ionization vitality of 70 eV. The particle source temperature of mass spectrometer was set at 180°C and the deferral for dissolvable was 5 mins. The mass spectra filter extend was 30-400amu. GC top territories were utilized to register the rate structure of an example without utilizing remedy factors. The distinguishing proof of fundamental oil constituents was at first completed by contrasting mass spectra of a compound and NIST-2008 MS library. As a second step, the maintenance lists of isolated mixes were resolved in respect to the maintenance times of standard C9 to C24 n-alkanes at a similar GC-MS parameters utilized for the basic oils. The figured maintenance lists of mixes were contrasted and the distributed information for the assurance of elution request and distinguishing proof of mixes. At last, the distinguishing proof of compound was accomplished by co-infusion

Bioactivity assessment of *Pinus* needles leaves

Antioxidant activity

The antioxidant activity of plant extracts was carried out according to method earlier reported (30) Therefore, for determination of antioxidants, following bioassays were used.

DPPH scavenging bioassay

DPPH scavenging activity was done according to method reported by (30) with some modifications. This method was based on the ability of antioxidant to scavenge 1, 1-diphenyl-2-picryl hydrazyl (DPPH) action radical. In a falcon tube, 100 µL of the sample solution was taken and then 4mL of DPPH solution (0.1 mM) was added and mixture was vortexed vigorously. Then the mixture was incubated with Aluminium foil to avoid heat exposure in the dark for 30 min at room temperature. UV-Vis spectrophotometer was used to measure the absorbance of the solution at 517 nm. Percentage inhibition was calculated by following formula.

$$\% \text{ Inhibition} = A_{blank} - A_{sample} \times 100 / A_{blank}$$

ABTS scavenging bioassay

The ABTS radical scavenging bioassay was carried out by already reported method (29,30). The ABTS radical cation was prepared by mixing 5 mL of a 7 mM aqueous ABTS solution to an about 88µL of 140 mM potassium per sulphate by making the final concentration of solution to be $0.7cm^{-1}$. The leaves extracts were prepared in ethanol to a volume equal to 25 µL. 10µL of sample solution was added to the reaction mixture and final percentage reduction in absorbance was measured at 730nm. The expected final absorbance was assumed to be 20-80% decreased as compared to the initial absorbance of reaction mixture.

Scavenging of H_2O_2

Scavenging of H_2O_2 was determined by using method reported (31). Briefly about 4 mM of H_2O_2 (0.6 mL) solution was added to 4 mL of extract and incubated for 10 min. The absorbance of solution was measured at 230 nm against a blank solution via spectrophotometer method. Free radical scavenging potency as determined from %age of H_2O_2. Lower value of H_2O_2 indicated strong free radical scavenging activity of leaves extracts (30,31).

Anti-mycobacterium activity

The two drug resistant strains of *Mycobacterium tuberculosis*, bg 206 and bg 1972 along with a sensitive strain H37Rv were used in this experiment. Inoculum of all strains were prepared by using method described (9). From dilution (10^{-2}) of each strain of *Mycobacterium tuberculosis* suspensions, about 60µl was streaked on the LJ slants using loop with 3mm external diameter. The crude extract at various concentrations of 5 mg/ml, 10 mg/mL and 50 mg/mL were separately incorporated in

the medium and this process was performed for all extracts. Antimycobacterial activity was done according to the methods (9, 30).

Brine shrimp cytotoxicity bioassay

Hatching of shrimps

Brine shrimp eggs were hatched in a shallow rectangular dish (22 x 30 cm) filled with artificial sea water that was prepared with a commercial salt mixture and double distilled water. A plastic divider with 2 mm holes was clamped in the dish to make two unequal compartments, the eggs (50 mg) were sprinkled into larger compartment which was darkened while the smaller compartment was illuminated. After 48 hrs, the phototropic nauplii were collected by pipette from the illuminated side, which was separated by the divider from their shells (19,29). Twenty shrimps were transferred to each sample vial using pipette and 5mL artificial sea water was added. The nauplii can be counted in the stem of pipette against a lighted background. A drop of dry yeast suspension (3 mg in 6mL artificial sea water) was added as food to each vial (Solowey *et al*., 2014). The vials were maintained under illumination. Survivors were counted with the aid of 3 magnifying glass and after 24 hrs percent death at each dose and control were determined. In each case, where control deaths occurred, the data were corrected using Abbott's formula:

$$\% \text{ Death} = [\text{ Test} - \text{Control}] / \text{Control} \times 100$$

Statistical analysis

Data obtained were analyzed statistically by using one way ANOVA and results were expressed in form of mean, standard deviation and percentage values.

RESULTS AND DISCUSSION

Samples of *pinus* needles leaves and *pinus* resins were chemically analyzed for various parameters and results were mentioned in following sections

Assessment of oil contents of needles leaves and resins

The extraction of oil was carried by distillation method by using Soxhlet

apparatus and oils was extract from needles of *Pinus Roxburghii,* and *pinus* resins and results were mentioned (Tables 1-2).

Table 1. Extraction of oils (%) from Needle leaves samples of *pinus* roxburgii

Samples	Methanol	Ethanol	Acetone	Hexane
Needle leaves 1	45.5 ± 1.2	41.5 ± 1.4	11.6 ± 0.9	8.6 ± 0.6
Needle leaves 2	42.4 ± 1.5	37.6 ± 1.2	3.2 ± 2.1	4.7 ± 0.8
Needle leaves 3	33.5 ± 2.3	31.4 ± 0.7	1.6 ± 1.4	1.91 ± 0.7

Mean ± S.D (n=3).

Table 2. Extraction of oils (%) from resins samples obtained from stems of *pinus* roxburgii

Samples	Methanol	Ethanol	Acetone	Hexane
Resins 1	51.5 ± 1.5	48.3 ± 1.8	14.5 ± 0.8	12.7 ± 0.7
Resins 2	45.6 ± 1.1	41.5 ± 1.1	6.5 ± 2.3	3.9 ± 0.9
Resins 3	36.4 ± 2.1	31.0 ± 0.9	2.7 ± 1.1	1.90 ± 0.6

Mean ± S.D (n=3).

Table 3. Extraction of Biodiesel and glycerin after trans esterification process

Samples	Biodiesel (%)	Glycerin (%)
Methanol	10.5 ± 1.2	15.6 ± 3.2
Ethanol	8.2 ± 1.6	11.3 ± 1.5
Acetone	6.3 ± 1.1	7.2 ± 1.5
Hexane	7.4 ± 1.6	9.3± 1.6

Mean ± S.D (n=3).

Conversion of oil into Biodiesel and glycerin

Results regarding biodiesel and glycerin production, after trans esterification process of crude oil are given into table3. Higher quantity of Biodiesel was produced when transesterfifction was carried out by using methanol and sodium hydroxide, 10.5 ± 1.2% / 100 ml of crude oil . Whereas 15.6 ± 3.2 % of glycerin was produced when 100 ml of crude oil was used for transesterification process.

Pure glycerin / glycerol has various uses in food, pharmaceutical, medical and personal care industries as reported by different research workers (31,32).

Physical and chemical analysis of Biodiesel

Conformity tests of Biodiesel were carried out and results of pH, acidity, iodine values and saponification values are given in table 4

Table 4. Various parameters of Biodiesel

Parameter	**Results**
Color	Pale yellow
pH	6.5
Acid value (mg of NaOH/g of oil	1.3 ± 0.01
Iodine (mg of I2/g of oil)	81.5± 0.2
Saponification value (mg of KOH/g of fat	175± 1.18
Refractive index	1.46± 0.01

Mean ± SD

Analysis of Phyto chemicals

Quantitative analysis of needle leaves and resins are given in (Table 3). According to results higher quantity of flavonoids fallowed by total phenols and tannins were present in needles leaves. Whereas lower quantity was obtained from pinus resins (9).

Table 5. Analysis of methanolic extracts of *Pinus* needle leaves and resins of Pinus *Roxburghii* (chir pine) for different phytochemicals

Constituents	**Resins**	**Needles leaves**
Total phenol mg/g	3.28±0.72	7.26±1.52
Total flavonoids mg/g	4.15±1.38	14.53±2.45
Total tannins	2.87±0.54	4.36±1.23

Mean ±SD (n=3)

Analysis of oil by GC- MS

Oil extracted from various samples of pinus needles leaves and resins was analyzed by GC –MS. It was found many fatty acids were present (Table. 5).

Table 6. Fatty acid contents of oil from *Pinus* resins analyzed by GC-MS

Name	RT	% Compounds
1-DODECANOL, 2-OCTYL-	8.801	0.008304
TETRAPENTACONTANE, 1,54-DIBROM	11.042	53.84524
1(2H)-NAPHTHALENONE, 6-(1,1-DI	21.181	41.54379
1-DODECANOL, 2-HEXYL-	32.356	0.013741

There were four compounds of fatty acids detected from oil of by GC-MS. However, two compounds like tetrapentacontane (53.84 %) and Napthahalenone (41.5%) were present with higher concentration (Table 6, 7; Fig.2).

Table 7. Fatty acid contents of oil from needles leaves analyzed by GC-MS

Name	RT	Area	%Composition
OCTADECANE, 1-CHLORO-	22.422	11131664384	96.51861
1-DODECANOL, 2-OCTYL-	33.801	25381554	0.220074
1-DODECANOL, 2-HEXYL-	34.807	6787248.5	0.05885
HEPTACOSANE, 1-CHLORO-	36.943	10392788	0.090112
17-PENTATRIACONTENE	37.098	3289870.25	0.028525
1-PENTACONTANOL	37.538	744749.688	0.006457
TETRAPENTACONTANE, 1,54-DIBROM	42.08	53473556	0.46365

There were seven compounds of fatty acids detected from oil of *pinus roxburgii* by GC-MS. However, compound like OCTADECANE, 1-CHLORO- (96.51) is present with higher concentration (Tables 6-8; Fig.2).

Figure 2. GC – MS analysis of pinus needl leaves

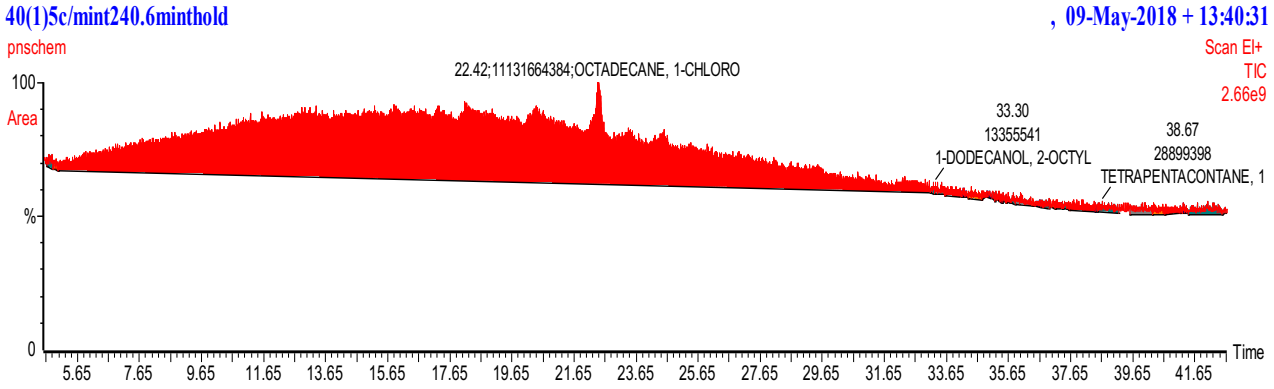

Table 8. Number of Carbon atoms of fatty acid found in organic compounds

Fatty acid	P. R (%)	C
C18 :O	96.5	0.08
C 15: 0	0.64	
C 20 :0	0.22	53.84
C 27:0	0.31	0.13
C35: 0	0.62	-
C 54: 0	0.46	-
C8 :0	-	41.54

Bioactivities of *Pinus* needles leaves

Antioxidant activities of leaves extract

In order to find out antioxidant activities, the extracts from needles of Pinus were employed for the DPPH, ABTS and H_2O_2scavenging bioassays. The scavenging activity of the needles extracts was much greater in H_2O_2 scavenging assay as compared to ABTS and DPPH scavenging assay (Tables 9-10).

Table 9. Antioxidant activity of leaves extracts of *Pinus Roxburghii* (chir pine) (IC50 values µg/ml)

Extract 100 µg/ml	DPPH	H_2O_2	ABTS
Ethanol	45.17±3.26	58.54±5.26	45.32±2.81
Methanol	25.38±4.15	42.56±3.15	39.46±2.28
N hexane	52.18±1.36	61.52±4.85	48.24±1.35

Ascorbic acid	9 .65±2.52	7.62 ±1.36	16.25±2.38
Gallic acid	6.34±1.32	5.65±1.25	8.26±1.36

Means ± SD, (n = 3).

Table 10. Antioxidant activity of Pinus *resins* extracts (IC50 values µg/ml)

Extract 100 µg/ml	DPPH	H_2O_2	ABTS
Ethanol	36.17±1.25	45.69±4.15	39.45±2.16
Methanol	27.28±1.48	38.21±3.17	32.89±4.36
N hexane	45.16±1.51	48.93±6.53	53.27±3.25
Ascorbic acid	12.29±1.43	9.12 ±2.36	12.35±1.25
Gallic acid	8.35±1.36	7.68±1.25	8.24±1.32

Means ± SD, (n = 3), whereas ^{a} = $p<0.01$, Þ= $p<0.05$.

Antimycorbaterium activity of pinus needles leaves

Medicial plants offer a hope for developing alternate medicines for the treatment of TB. The present study was carried out to evaluate in vitro anti-tubercular activity of different extracts of Pinus needles leaves extracts against different strains of mycobacterium tuberculosis (Tables 11- 12). Furthermore, results obtained in our study showed higher percentage of inhibition results reported by other authors including (13).

Table 11. Anti-tuberculosis activities of Pinus *needles leaves* extracts on LJ Media

Extracts	Isolates	Mean CFU on media				Percentage Inhibition		
		Control	5mg/ml	10mg/ml	50mg/ml	5mg/ml	10mg/ml	50mg/ml
Methanolic leaves extracts	H37Rv	140	36	2	0	70	99	92
	bg 206	150	49	23	0	65	85	82
	bg 1972	130	74	51	0	40	61	80

Methanolic leaves extract	H37Rv	140	30	0	0	75	100	85
	bg 206	150	45	20	0	72	87	82
	bg 1972	130	65	50	0	52	62	80
Methanolic leaves extracts	H37Rv	140	80	76	20	45	46	65
	bg 206	150	90	60	35	35	60	70
	bg 1972	130	86	64	40	30	51	50

Table 12. Anti-tuberculosis activities of *resins extracts* on LJ Media

Extracts	**Isolates**	**Mean CFU on media**				**Percentage Inhibition**		
		Control	5mg/ml	10mg/ml	50mg/ml	5mg/ml	10mg/ml	50mg/ml
Methanolic leaves extracts	H37Rv	140	36	2	0	74	99	100
	bg 206	150	49	23	0	67	85	100
	bg 1972	130	74	51	0	43	61	100
Methanolic leaves extract	H37Rv	140	30	0	0	79	100	100
	bg 206	150	45	20	0	70	87	100
	bg 1972	130	65	50	0	50	62	100
Methanolic leaves extracts	H37Rv	140	80	76	20	43	46	86
	bg 206	150	90	60	35	40	60	77
	bg 1972	130	86	64	40	34	51	69

Cytotoxiciy assessment of pinus needles leaves

Assessment of cytotoxic behavior of medicines pants used for drugs development are important which indicates that plant extracts is how much toxic to any cell. Results of brine shrimp cytotoxicity assay is given in tables (13, 14).

Table 13. Cytotoxicity screening of methanolic*P. Roxburghi* pine needles extracts (µg/ml)

Concentration (ug/ml)	**Total nupuli**	**Live after 24 hours**	**Death after 24 hours**	**% of death**
10	18	17	1	5.56
100	20	14	6	30.0

300	20	12	8	40.0
600	19	10	9	47.37

Values are Mean±SD ,(n=3) and significantly different (P<0.05); positive control are saline sea salt

Table 14. Cytotoxicity screening of methanolic pine resins extracts (µg/ml)

Concentration (ug/ml)	Total nupuli	Live after 24 hours	Death after 24 hours	% (death)
10	18	15	3	16.66
100	20	12	8	60
300	20	8	12	40.0
600	19	6	13	31.50

Values are Mean±SD ,(n=3) and significantly different (P<0.05); positive control are saline sea salt

People around the world use herbal supplements and medicines due to their beneficial effects on human health (8). Bark, needles, pollen and other parts of numerous pine species have been used for many years and proven to constitute excellent raw materials in the production of goods (4).

Conifer shoots are virtually unused as a food ingredient, despite their common availability in many parts of the world. The addition of Pinus extracts increases the antioxidant potential of juices and dairy products as regard to juices, which indicates that it may also serve as an ingredient providing flavour and aroma [15]. In the case of the addition of pine extract to bread and meat, the substance acted as a shelf-life extender by inhibiting the growth of bacteria and oxidisation of fats (6). The seeds of pinus have higher energy value due to a high fat content (21). The seeds also generally have the highest content of the tested nutrients, excluding vitamin C, which is higher in the conifer needles. The seeds of pinus can be a good source of Mg, P and especially Zn (24).

Macronutrients affect biochemical processes, physiological responses and yield quantity (17, 25). When it comes to macronutrients, their role in plant organisms includes many life processes that determine plant functioning (24,26). Therefore, it is very difficult to clearly indicate a specific role of elements because they act in a complex way.

The role of micronutrients, on the other hand, is more specific, as it is related to specific, (27, 28). Nutrient deficiency results in various disorders in terms of the normal growth and development of the plant

It was reported earlier that Pine essential oils contain more than 50 ingredients (19). Their concentrations vary depending on the plant variety, crop, distillation method and part of the plant (Tables 5-7). Studies have shown that these phytochemicals exhibit diverse biological activity, which contributes to their various uses and applications. They can be used as fungicides, flavours and fragrances, as well as antiviral and antimicrobial agents. The uses of α- and β-pinene go beyond therapeutic and nutritional applications. They are versatile compounds that are used in polymer synthesis (20).

Polyphenols are essential secondary metabolites that allow plants to grow and develop. They also protect plants from insects and other factors (13). Polyphenols found in plants are involved in functions related to sensory properties such as colour, bitterness and sourness (13,14). Simple phenols and flavonoids correspond to most natural phenolic substances. Moreover, flavonoids belong to the most common group of these compounds. The demand for phenolic acids is very high in many industries well-defined life processes in the plant and to plant growth

because they are used as precursors to other important bioactive molecules that are regularly needed for therapeutic and cosmetic purposes, as well as for food industry. Phenolic acids are also commercially available as dietary supplements (15). Various parts of a pine (needles, seeds, bark and cones) and different solvents can be used to extract polyphenols. The pine bark is the best-examined part.

mechanism of action of polyphenols is strongly associated with their antioxidant activity and reduction of reactive oxygen species in the human body (14,15). Furthermore, the health-promoting properties of plant polyphenols include anti-inflammatory, anti-allergic, anti-atherosclerotic, anticoagulant and antimutagenic effects (16). The daily intake of polyphenols among the general population ranges from 0.1 to 1.0 g per day. Fruit, vegetables, herbs, spices, coffee, tea and wine are the main source of polyphenols (17). The assessment of biological activities of the phytochemicals to have beneficial therapeutic capacity for curing human from various illness. Therefore, variety of chemical compounds obtained by the tested plants impart significant anti proliferative,

cytotoxic, and anti-tuberculosis activities. In the present study, the plant extracts were assessed for the presence of flavonoids, total phenolics and tannin. The study indicates that *pinus* needles leaves and *pinus* resins. According to results pinus needles tree and resins are comprised significant number of total phenols and tannins (Table. 4-5). Phenols constitute the largest group of secondary metabolites, varying in size from a simple structure with aromatic ring to complex ones. Phenolic compounds, ubiquitous in plants are an essential part of human diet and are of considerable interest due to their antioxidant properties. The presence of significant amount of these important phyto-constituents bestow the plant with high medicinal activities like free radical scavenging and antimicrobial activities. The variation in quantity of phytochemicals may be dependant upon both the chemical structure and the number of individual compounds in plant material (7).

By comparison of chromatogram of GC-MS, the area of peaks of compounds was calculated and other required information was obtained by NIST library data base (Tables 6-8). The prominent peaks in the chromatogram of essential oil of indicates that *pinus* needles and resins consist of some important fatty acids (9). It was observed that the variation in chemical constituents of essential oils might be due to the variation in contents of leaves and resins because physical structures of needles leaves are different from resins (17). The composition of oil directly affects the effectiveness of biological activities which have displaces difference in its constituents depending on the growing area whereas chemistry of oil is complex and variable The seasonal changes may account in the variation in chemical constituents of plants grown at different parts of the world (24).

Chemical constituents with antioxidant activity present in plants determine the role of plants in prevention of many degenerative diseases. The human food supplements including herbs, contain higher number of compounds that are capable of deactivating free radicals (13). The plant extracts were assessed for their antioxidant potential by three methods that includes DPPH scavenging, ABTS scavenging and H_2O_2 scavenging bioassays. The methanolic extracts of the plants showed significant scavenging of free radicals. By comparing DPPH and ABTS bioassays results. It was assumed that the antioxidant potential of the three plant extracts is much increased in case of DPPH bioassay as compared to ABTS bioassay. The antioxidant potential of *P.*

Roxburghii was higher as compared to resins extracts analyzed (Tables 9-10).

DPPH and other scavenging bioassay revealed that free radical scavenging potential was present in plant extracts has been reported in literature by many authors might be significant antioxidant agent due to its excellent antioxidant activity for Reactive oxygen species (ROS). ROS and reactive nitrogen species (RNS) are some forms of activated oxygen and nitrogen respectively, which include free radicals such as superoxide ions, hydroxyl and nitric oxide radicals as well as non-free radical species such as hydrogen peroxide (H_2O_2), and nitrous acid (HNO_2). ROS and RNS have been the cause of more than 100 diseases which includes malaria, AIDS, heart diseases, stroke, diabetes and carcinogenity. It was reported thatpinus needles leaves have antioxidant as well as antimicrobial activity which may be attributed to the presence of various active secondary metabolites (27).

Similarly in the current research work, has shown a considerable amount of antioxidant potential. However the studies conducted in the other parts of world revealed the good antioxidant potential and anti-inflammatory activity of *pinus needles leaves* .

Scavenging of H_2O_2 displayed by extracts of plants was dose dependent. The total radical scavenging activity (superoxide and hydroxyl radical) of pinus needles was found to be quite significant and showed effective scavenging activity in dose dependent manner and suppressed the production of H_2O_2 at the dose concentration 300 µg/mL which is a characteristic of chain-breaking antioxidants and has been observed in oxidation of linoleic acid emulsion with extract. Flavonoids, phenolic acids and phenolic diterpenes are the examples of phenolic components with antioxidant properties. Scavenging of extracts may be characterized to phenolic content, which is capable of donation of electron to H_2O_2, thus nullifying to water. Although reactivity o H_2O_2 is not much, even then it can periodically be cytotoxic by ascending hydroxyl radicals in the cells. Expulsion of H_2O_2 by food stuffs is too much necessary (20).

The selected herbs have been used traditionally as a remedy for respiratory diseases like bronchitis, sinusitis, tuberculosis and common cold (21). These activities are mostly due to presence of essential oils. The results indicates that essential oils and other phyochemicals present in plant extracts has exhibited significant antibacterial activity (Tables 11-12). Therefore it is assumed that due to

presence of essential oils, phenols and flavonoids . Extracts of *Pinus* needles leaves as well as *pinus* reins might be good remedy against infection causes by mycobacterium tuberculoses. All the tested extracts are antibacterial in nature and results obtained are in accordance with the reported findings (25). Due to multidrug resistant strains of various bacterial strains new medicines are required to overcome tuberculosis in human population. Various plants have some active secondary metabolites those have wide range of application against such human disorders. There is need of continuous development of new and efficient methods to determine the susceptibility of isolates of mycobacterium tuberculosis in search for new novel antimycobacterial agents from natural products of plants sources. Therefore, in present study various solvents extracts of *pinus* needles leaves have provide remarkable anti mycobacterium activities indicating its usefulness control aliments of tuberculosis in human population (26). Tuberculosis (TB) is an infectious disease mostly caused by mycobacterium tuberculosis mostly effects on lungs but some cases also effect other parts of body. According to literature about one-third of world population is suffering TB, that is increasing at rate of 1 % per years (19).

Practice for uses of pinus needles leaves as antimicrobial agent is taking since many decades . It was reported by many research workers that smell of pinus needles are important for respiratory tract system (20). According to results of anti-mycobacterium activity of pinus needles were proposing. The increasing incidence of infectious diseases, severe side effects related to the intake of many antibiotics and the development of antibiotic resistance substantiate the growing interest in the identification of new antimicrobial compounds, both natural and synthetic agents (24).

Plant resin has been applied to treat diseases in folk medicine for thousands of years. It was also used in the pharmaceutical industry before the introduction of modern antibiotics. The antimicrobial activity of extracts, oils and resins from trees of the Pinus genus may be related to various organic Plants

The discovery of biological effects of the chemical compounds available Pinus suggests that they may be applied in the creation of environmentally friendly and biocompatible pharmaceuticals (32).

Cytotoxicity refers to the ability of certain chemicals to destroy the living cells in the body. By a cytotoxic compound, healthy living cells either induce necrosis (accidental cell death) or apoptosis (programmed cell

death). Brine shrimp lethality bioassay used for *pinus* needles leaves and resins indicates that all the plant extracts are very less toxic and are suitable to be used in folk medicine, which also indicates importance of *these* extracts for its application in pharmaceutical industry for development of drugs. The toxicity of extracts was assessed which revealed that all of the plant extracts were less toxic towards shrimp's napulii at higher concentration (600 µg/mL) which confirmed their efficiency to be used in preparation of future drugs (31,32).

CONCLUSION

Various parts of *pinus* tree contains different bioactive compounds. Green and dried Needles leaves, resins and cones are important sources of food materials as well as material required domestically. The pine bark extracts are commercially available, there is no universal method of extraction that is suitable for all phenols. Depending on the goal of extraction, an individual examination should be performed to ensure the most appropriate extraction procedure. Regardless of the solvent, method, pine species and plant part used, all pine extracts contain a high number of polyphenols. Nevertheless, individual compounds are characterized by different concentrations, types and levels of their bioactivity. There are few studies on the identification and even fewer studies presenting the quantitative determination of individual polyphenols contained in pine extracts. Pine tree extracts exhibit several described biological activities that may be beneficial to human health. The available examples of the application of pine elements in food are promising. Pine tree extracts, syrups and other intermediates may be components that impart functional properties, extend the shelf life and assign desirable qualities to food products. Pine extracts and oils exhibit great potential as formulation ingredients for food, cosmetic and pharmaceutical industries. *Pinus* resins is natural fuel that can be converted into other fuels like biodiesel etc to meet future requirements of energy. The reuse of residual pine elements is still limited compared to its potential. In this case, it is necessary to conduct more research to find and develop new products and applications of pine residues and by-products.

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SIGNIFICANCE OF VACCINES IN THE DETERRENCE OF DISEASES; AN UPDATE

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ABSTRACT

Vaccination is a biological preparation that gives active acquired immunity against a specific infectious disease. Vaccines are produced from attenuated form of microbes, its toxins and surface protein of microorganisms which causes disease. There are four types of vaccines (a) live attenuated vaccines (b) inactive vaccines (c) subunit recombinant polysaccharide and conjugated vaccines as well as (d) toxoid vaccines. Therefore, vaccines can be in the form of adjuvant, valence, excipients, and preservatives. There are many types of viral vaccines for different disease such as measles, mumps, rubella, vaccinia, varicella, zoster etc. The current example of viral disease is COVID 19 epidemic, which is causing serious health conditions in human population throughout the world. There have been 222,788,994 confirmed cases of COVID, with 4,600327 deathsand recorded cases were 199,314,577 as reported by WHO on September 8, 2021. To deal with this problem, experts of viral diseases from all over the world, particularly in wealthy countries, are frantically trying to create vaccines that could have the ability to treat coronavirus sufferers. However, a large portion of the world's population continues to wait for their fantasy medications to arrive in the markets. This review is aimed to explore the importance of vaccines for the immunization against various infectious diseases including the most promising anti-COVID-19 vaccine clinical trials as well as the mechanism of vaccine development and vaccination process in a biological system.

Keywords; Vaccine, COVID-19, Immunization, T-lymphocytes, DNA vaccines, RNA genome

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INTRODUCTION

The health of a country's population has an impact on its economic progress. Capital, health, and education are among the most important variables in a country's

development. Investments in the domains of health and education would hasten economic development (Ahmad,2021). Individuals' contributions to production and growth will increase if they are healthy. Every year many people get infectious diseases round the world so they may need vaccination to prevent these ailments before reaching to an incurable state. The vaccines train immune system to recognize and clear out the virus. The immune system of human body builds this protection after getting proper vaccination. Vaccine development and manufacture are both expensive and vulnerable to market failure. Many of the infectious diseases for which a vaccine is needed, such as HIV, malaria, and TB, are mostly found in developing nations. Because there is minimal financial potential, pharmaceutical and biotechnology companies have little motivation to produce vaccines for these illnesses. Financial rewards are generally limited, and financial and other risks are high, even in more prosperous countries. Too far, most vaccine development has relied on "push" financing from the government, universities, and non-profits. Many vaccinations have shown to be both cost-effective and helpful to the public health. Vaccine research and development is done out by a series of smaller organizations (Ahmad, 2021).

Mechanism of vaccine development

VLPs (viral like particles) are highly organized, repeating structures with a high density of viral capsid proteins. This high concentration of capsid proteins results in many conformational viral epitopes, which can trigger powerful immune responses. In the absence of any infectious nucleic acids from the virus, VLPs are generated by the self-assembly of viral capsid proteins (Figure 1). As a result of their complete inability to reproduce, they may be a safer alternative to the attenuated viruses typically used for vaccination (Dongarwar and Hamisu, 2021). Even in the absence of an adjuvant, VLPs have been demonstrated to trigger powerful immune responses. Structural proteins in the virion are often organized in a tight and well-ordered shape, which is thought to be recognized as a PAMP. As a result, delivering viral antigens in multimeric shape and as virus-like particles is one method to improve their immunogenicity (VLPs). VLPs made from enveloped and non-enveloped viruses can be utilized to immunize against the same virus or modified to include epitopes from a different pathogen. VLPs are considered very safe

since they contain no genetic material, in addition to having greater immunogenicity. For decades, recombinant viruses have been employed as vectors for protein expression and immunization. The number of viral families being investigated as vaccine vectors is far too long to be detailed, and the subject has lately been discussed elsewhere. Viruses may be modified to improve their safety and immunogenicity by removing virulence factors, altering tropism by switching envelope proteins, and boosting coding capacity by removing non-essential genes. The antigen is produced in the context of a real viral infection, which triggers innate immune responses necessary for the full development of adaptive humoral and T cell-mediated immunity (Koirala *et al*., 2020). Competition of immune-dominant antigens from the vector or loss of effectiveness in the face of pre-existing immunity against the vector are potential drawbacks. The non-structural protein NSs is a key virulence factor that regulates the immunological response of the host, although it is not necessary for cell culture replication. Several organizations have produced viruses missing NSs through applying reverse genetics to attenuated strains and demonstrating safety and immunogenicity in mice and lambs.(Oyarzún and Kobe, 2016)

Recombinant proteins and synthetic peptides

Delivering a viral antigen made by recombinant techniques or chemical synthesis is a safe way to trigger immune responses. Recombinant protein vaccines can have other benefits in addition to safety: First, manufacturing does not need pathogen manipulation, which eliminates the possibility of inadvertent escape as well as the challenges of bio-safety and bio-containment. Second, even with minimal information about the disease, vaccine candidates can be developed. Third, subunit vaccinations can be utilized to circumvent the immune system's inherent preference for highly variable epitopes and steer immune responses toward conserved and widely protective epitopes. Fourth, because specific antigens elicit responses distinct from those elicited by natural infection, these vaccine techniques could be employed as DIVA (Differentiating Infected from Vaccinated Animals) vaccines with a serological test(Leroux-Roels*et al*., 2011).The main disadvantage of subunit vaccines is that isolated proteins or peptides are usually poor immunogens because they

do not recognize Pathogen-Associated Molecular Patterns (PAMPs) and thus do not activate innate immune responses, which are necessary for the full development of acquired immunity. To boost immune responses to conserved epitopes, they must be delivered in an immunogenic form and/or be accompanied with a powerful agonist.(Wallis *et al*., 2019)

Nucleic acid vaccines

DNA vaccines provide a number of potential benefits for vaccinations against new viruses: plasmids expressing a viral antigen can be made quickly, even if only a partial sequence of the pathogen is known. Antigen generates both humoral and cell-mediated immune responses when it is produced in vivo. DNA preparations are more stable than other forms of vaccines and can be made in large quantities in a short amount of time at a lower cost, both of which are important qualities for a vaccine that must be utilized in distant places. Furthermore, DNA vaccines are regarded to be extremely safe, are ideal for DIVA applications, and are immune to anti-vector immunity (Maiyegun*et al*., 2021). The inherent poor immunogenicity of DNA vaccines is the primary impediment to their development. In prime-boost techniques, DNA vaccines are widely employed in conjunction with other vaccination platforms. Replicon vaccines are made up of faulty RNA genomes that can replicate and express encoded proteins but not form infectious virus particles. These plasmids can be utilized to encode a viral antigen, which can result in antigen-specific humoral and cellular immune responses. These findings sparked a massive amount of research into DNA-based vaccines for a variety of diseases, including influenza, HIV, and lymphocytic choriomeningitis virus (LCMV).DNA vaccines are more cost-effective than protein, whole cell, or viral vectors in practice because DNA can be generated using simple scalable chemistry or produced in large quantities in bacteria. Due to the limitations of DNA vectors, RNA-based vaccinations have gained popularity in recent years. They are low-cost and can be mass-produced quickly, similar to DNA-based vaccinations.(Oyarzun and Kobe, 2016; Leitner,2020)

However, the instability of RNA and ineffective in vivo distribution have traditionally limited its utility. To improve the intracellular stability of RNA molecules, several structural modification approaches have been used. Because RNA, unlike DNA, does not require targeting to and entry into

the nucleus, the fundamental obstacle that RNA vaccines must overcome is cell entry. This can be addressed by including polycationic carrier molecules in the formulation, which can condense and preserve the RNA while also facilitating its rapid cellular uptake (May, 2005).

Conjugate vaccines

Vaccines containing live, attenuated, or inactivated pathogens contain a variety of antigens, both polysaccharide and protein based. However, it is possible that only a limited number of them are needed to elicit protective immunity. The understanding that each protein has hundreds of potential immunogenic epitopes, not all of which are required has extended this reasoning to proteins (May,2005). Peptide-based vaccines have sparked interest because of this. Antigenic epitopes on a protein, on the other hand, are more than just a sequence of amino acids since the peptides utilized must imitate the immunogenic epitope's shape in the native protein. Computational modeling has proven to be a useful tool for locating and mapping the conformation of immunogenic epitopes within proteins. Because peptide or polysaccharide-based vaccines are less immunogenic than those found on a pathogen's surface, they require the addition of an adjuvant when administered (Sing *et al*.,2021). Another option is to conjugate the antigen to a second 'helper' protein or polysaccharide that has been shown to boost immunogenicity; however, this may cause the immune response to be diverted toward the helper molecule. Approaches to circumvent this difficulty include careful matching and orientation of the target and helper sections of the vaccine, or spatial segregation of the two subunits using carrier systems like as liposomes (Metz *et al*., 2009).

Cellular vaccines

Attempts to employ a similar strategy to vaccinate against cancer have been made due to the history of success of immunization using live attenuated viruses, inactivated viruses, or bacteria. To generate an immune response against specific types of malignancies, attenuated tumor cells have been given. There have been two types of whole cell vaccines used: autologous and allogeneic. Cancers such as lung, colorectal, melanoma, kidney, and prostate cancer have all been studied with autologous cell vaccines. Autologous cell vaccines, on the other hand, are confined to a few types and stages of cancer since they require a significant amount of the patient's tumors for

preparation (Sorochi*et al*., 2021). Many whole cell vaccines have been genetically engineered to induce the expression of cytokines, chemokine's, and co-stimulatory molecules in order to boost immune activation. Another type of cellular vaccination makes use of the patient's own immune cells, specifically dendritic cells. Dendritic cell vaccines are made by loading tumor-associated antigens or nuclei into a patient's autologous dendritic cells while they are being treated with immuno-adjuvants. In clinical studies, dendritic cell vaccines have been tested against prostate, melanoma, kidney, and glioma tumors. This vaccine regimen necessitates the collection of the patient's peripheral blood mononuclear cells, followed by cell culture processing and reinfusion, both of which are time-consuming and costly procedures. While these cell-based techniques are intriguing, they do not appear to contribute to the transition away from live and attenuated vaccines and toward vaccines with lower complexity and production costs that are better suited to treating large populations while decreasing health-care expenditures.(Oyarzún and Kobe, 2016)

Recombinant bacteria as vaccine vectors

Bacteria can be employed as vectors for the in vivo delivery of antigens or DNA, in addition to being widely used to manufacture recombinant subunit vaccines. The low cost and ease of scaling-up production, the availability of well-characterized attenuated strains, the vector's activation of innate immunity, and the efficient delivery to antigen-presenting cells are all potential advantages of this platform. Listeria, Salmonella, Lactococcus, and Bordetella are among the genera being investigated as vaccine vectors. Recombinant bacteria can be utilized as live vaccines, inactivated germs, or even bacterial ghosts with no cytoplasm. In mice, recombinant *Lactococcuslactis* expressing the SARS-coronavirus N protein has been demonstrated to produce antibodies(Wallis *et al*., 2019).

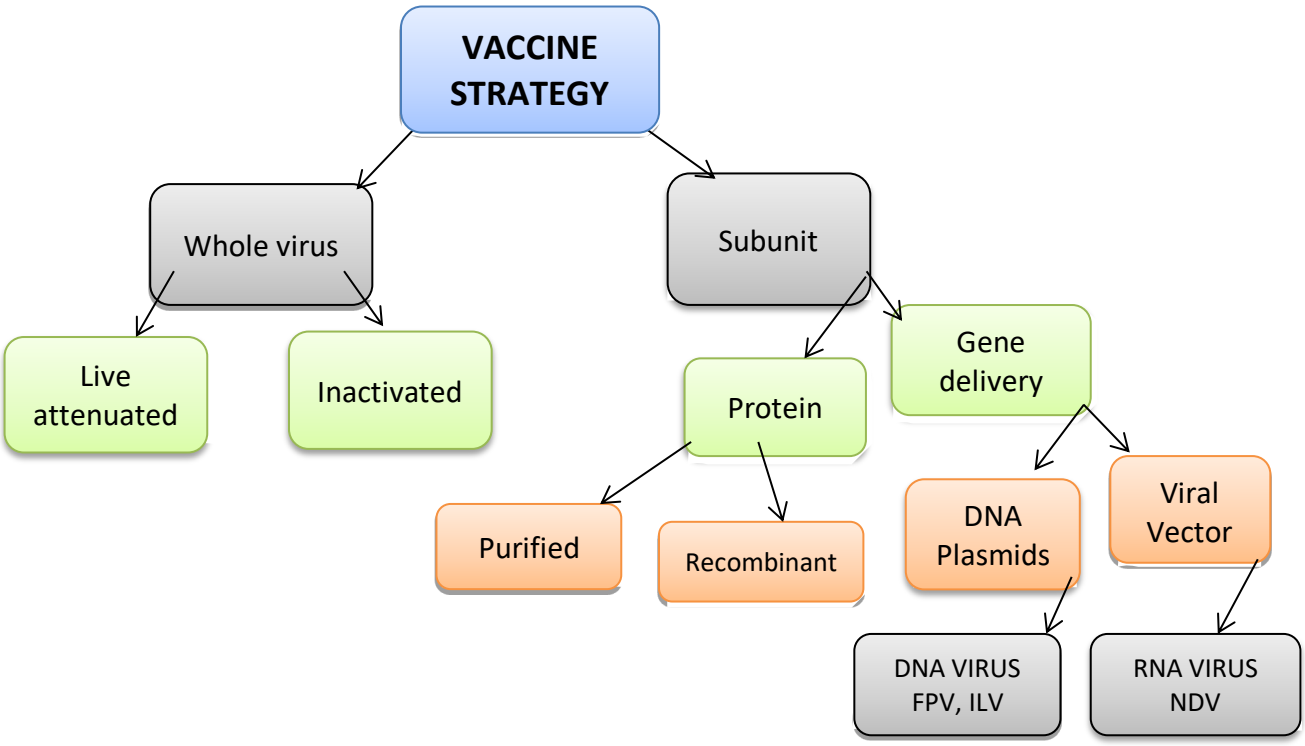

Figure1. Different steps forvaccine development

Immune Response to SARS-CoV-2 Virus

The immune system affects the severity of the COVID-19 disease. SARS-CoV-2 infection has an impact on both innate and adaptive immune responses. It has been described that SARS-CoV-2 enters the human body through physical barriers, such as respiratory tract, oral mucosa, and conjunctival epithelium. The dendritic cells, macrophages, and neutrophils represent the first line of defense, and their functions may be promoted by the production of type I and III interferons by SARS-CoV-2-infected epithelial cells. The adaptive T-cell- and B-cell-mediated immune responses are also presented in COVID-19 disease and, however, can be suppressed by SARS-CoV-2. In some cases, the innate immune cells may contribute to the excessive inflammation and, therefore, to the disease progression. The inability to reach control over the infection may result in dysregulated inflammatory responses that are potentially lethal. The IgM and IgG antibodies to SARS-CoV-2 are detectable within 1–2 weeks and began to decrease by 8 weeks. Several studies also reported that IgA response peaks earlier than IgM. The antibody response particularly leads to production of neutralizing antibodies to the S protein and to the nucleoprotein. S protein is also the main target of the majority

of newly designed vaccines. The magnitude of neutralizing antibodies positively cell responses were detectable in individuals recovering from mild COVID-19 who did not have detectable antibody responses to SARS-CoV-2. The effective vaccination may not eradicate the SARS-CoV-2 virus but may at least protect from severe and deadly forms of the COVID-19 disease. Current knowledge regarding the diverse aspects of SARS-CoV-2-immune system interplay shall be reflected in the vaccine design, including the selection of antigens, the vaccine platforms and adjuvants, the vaccination routes, and the dosage regimen. The key points of the SARS-CoV-2 vaccination strategies are discussed below. To date, over 80 clinical trials have been registered in the Clinical Trials database by the National Library of Medicine at the US National Institutes of Health; however, only 34 of them are active and recruiting (11 of phase I, 8 of phase I/II, 3 of phase II, 1 of phase II/III, and 11 of phase III). Moreover, 2 vaccine candidates have been approved for use by the US *Food and Drug Administration* (FDA) – BNT162/Comirnaty and mRNA-1273). BNT162/Comirnaty has been also permitted by the *European Medicines Agency* (EMA). The vaccination program with BNT162/Comirnaty has been recently initiated in many European countries.

correlates with the disease severity and the robustness of T-cell response. T-

Inactivated Vaccines

Inactivated vaccines are based on presenting the form of pathogen with a loss of disease-producing capacity. The virus cultivation occurs in cell lines that represent a substrate for the production of large quantities of antigen. Virus multiplication is often followed by a purification and concentration prior to the vaccine inactivation. Formaldehyde and beta-propiolactone are used in the majority of licensed human antiviral vaccines to inactivate the virus. Multiple doses or adjuvants are required to achieve sufficient efficacy of inactivated vaccines. To date, 4 inactivated vaccines have reached the phase III clinical trials and are currently under evaluation (#NCT04510207, #NCT04508075, and #NCT04456595).

DNA Vaccines

DNA vaccines deliver coronavirus's genes to the human cells. The vaccination principle depends on the DNA translocation into the cell nucleus where the transcription of the antigen is initiated and followed by a translation. DNA vaccines frequently use

plasmids as vectors. Depending on the route of vaccine administration (intramuscular, intradermal, and subcutaneous), either myocytes or keratinocytes are addressed. Nonetheless, antigen-presenting cells residing close to the site of application can be transfected directly by DNA vaccines as well. In such cases, the expressed antigens are loaded onto MHC I and MHC II molecules due to the cross-priming potential. The produced antigens are either released by exosomes or apoptotic bodies which lead to a recognition by antigen-presenting cells and further evolvement of humoral or cytotoxic immune responses. Different delivery devices are used to create a robust immune response. The main safety concerns imply a possible integration of transfected DNA into somatic and/or germ cells of the host. In such cases, a dysregulation of gene expression might occur and lead to various mutations. However, only extrachromosomal plasmids with a very low level of chromosomal integration are usually employed in the development of DNA vaccines. Furthermore, the majority of plasmids remain at the site of administration.

RNA Vaccines

Messenger RNA (mRNA) vaccines were first tested in early 1990s; however, their use was limited because of their instability. The mRNA encodes the genetic information to produce an antigen, and thus, RNA vaccines also lead to a production of coronavirus's proteins in vivo. The in vitro generation of an RNA vaccine includes a reaction of a DNA plasmid template and a recombinant RNA polymerase. In addition, a synthetic cap analog and a poly(A) tail are added to form a mature RNA sequence. The stabilization is further achieved by various transport systems (such as lipid nanoparticles, nano-emulsions, and cationic peptides) or methods enabling facilitated transfection (gene gun and electroporation). Conventional mRNA vaccines are based on the initiation of the transient antigen expression in the cytoplasm of the host cells. Another platform is represented by self-amplifying mRNA vaccines that contain both the genes coding the targeted antigen as well as the genes required for the self-replication (mostly RNA-dependent RNA polymerase). The conventional mRNA vaccines induce a prompt antigen expression, and the expressed antigens generate both humoral and cellular immune responses. In self-amplifying mRNA vaccines, a delayed antigen expression may prevail and limit the efficacy of the vaccine. Yet, the self-amplifying

mRNA vaccine platform reaches higher yields, and thus, an equivalent protection is abovementioned platforms are not capable of producing viral particles due to the lack of viral structural proteins. Moreover, neither conventional nor self-amplifying mRNA vaccines can integrate into the host genome. The mRNA-based vaccines were able to conferred at much lower doses. Regarding the safety profiles, the replicons of both induce production of functional antibodies with neutralizing properties in rabies, influenza, or Zika virus and also represent a promising vaccination strategy in the prevention against COVID-19 infection.

NOVEL VACCINE DESIGNS

Virus-like particles

VLPs (viral like particles) are highly organized, repeating structures with a high density of viral capsid proteins. This high concentration of capsid proteins results in many conformational viral epitopes, which can trigger powerful immune responses. In the absence of any infectious nucleic acids from the virus, VLPs are generated by the self-assembly of viral capsid proteins. As a result of their complete inability to reproduce, they may be a safer alternative to the attenuated viruses typically used for vaccination (Dongarwar and Hamisu, 2021). Even in the absence of an adjuvant, VLPs have been demonstrated to trigger powerful immune responses. Structural proteins in the virion are often organized in a tight and well-ordered shape, which is thought to be recognized as a PAMP. As a result, delivering viral antigens in multimeric shape and as virus-like particles is one method to improve their immunogenicity (VLPs). VLPs made from enveloped and non-enveloped viruses can be utilized to immunize against the same virus or modified to include epitopes from a different pathogen. VLPs are considered very safe since they contain no genetic material, in addition to having greater immunogenicity. For decades, recombinant viruses have been employed as vectors for protein expression and immunization. The number of viral families being investigated as vaccine vectors is far too long to be detailed, and the subject has lately been discussed elsewhere. Viruses may be modified to improve their safety and immunogenicity by removing virulence factors, altering tropism by switching envelope proteins, and boosting coding capacity by removing non-essential genes. The antigen is produced in the context of a real viral infection, which triggers innate immune responses necessary for the full

development of adaptive humoral and T cell-mediated immunity (Koirala *et al*., 2020). Competition of immune-dominant antigens from the vector or loss of effectiveness in the face of pre-existing immunity against the vector are potential drawbacks. The non-structural protein NSs is a key virulence factor that regulates the immunological response of the host, although it is not necessary for cell culture replication. Several organizations have produced viruses missing NSs through applying reverse genetics to attenuated strains and demonstrating safety and immunogenicity in mice and lambs.(Oyarzún and Kobe, 2016)

Recombinant proteins and synthetic peptides

Delivering a viral antigen made by recombinant techniques or chemical synthesis is a safe way to trigger immune responses. Recombinant protein vaccines can have other benefits in addition to safety: First, manufacturing does not need pathogen manipulation, which eliminates the possibility of inadvertent escape as well as the challenges of bio-safety and bio-containment. Second, even with minimal information about the disease, vaccine candidates can be developed. Third, subunit vaccinations can be utilized to circumvent the immune system's inherent preference for highly variable epitopes and steer immune responses toward conserved and widely protective epitopes. Fourth, because specific antigens elicit responses distinct from those elicited by natural infection, these vaccine techniques could be employed as DIVA (Differentiating Infected from Vaccinated Animals) vaccines with a serological test(Leroux-Roels*et al*., 2011) .The main disadvantage of subunit vaccines is that isolated proteins or peptides are usually poor immunogens because they do not recognize Pathogen-Associated Molecular Patterns (PAMPs) and thus do not activate innate immune responses, which are necessary for the full development of acquired immunity. To boost immune responses to conserved epitopes, they must be delivered in an immunogenic form and/or be accompanied with a powerful agonist.(Wallis *et al*., 2019)

Nucleic acid vaccines

DNA vaccines provide a number of potential benefits for vaccinations against new viruses: plasmids expressing a viral antigen can be made quickly, even if only a partial sequence

of the pathogen is known. Antigen generates both humoral and cell-mediated immune responses when it is produced in vivo. DNA preparations are more stable than other forms of vaccines and can be made in large quantities in a short amount of time at a lower cost, both of which are important qualities for a vaccine that must be utilised in distant places. Furthermore, DNA vaccines are regarded to be extremely safe, are ideal for DIVA applications, and are immune to anti-vector immunity (Maiyegun*et al*., 2021) . The inherent poor immunogenicity of DNA vaccines is the primary impediment to their development. In prime-boost techniques, DNA vaccines are widely employed in conjunction with other vaccination platforms. Replicon vaccines are made up of faulty RNA genomes that can replicate and express encoded proteins but not form infectious virus particles. These plasmids can be utilized to encode a viral antigen, which can result in antigen-specific humoral and cellular immune responses. These findings sparked a massive amount of research into DNA-based vaccines for a variety of diseases, including influenza, HIV, and lymphocytic choriomeningitis virus (LCMV).DNA vaccines are more cost-effective than protein, whole cell, or viral vectors in practice because DNA can be generated using simple scalable chemistry or produced in large quantities in bacteria. Due to the limitations of DNA vectors, RNA-based vaccinations have gained popularity in recent years. They are low-cost and can be mass-produced quickly, similar to DNA-based vaccinations.(Oyarzun and Kobe, 2016; Leitner,2020)

However, the instability of RNA and ineffective in vivo distribution have traditionally limited its utility. To improve the intracellular stability of RNA molecules, several structural modification approaches have been used. Because RNA, unlike DNA, does not require targeting to and entry into the nucleus, the fundamental obstacle that RNA vaccines must overcome is cell entry. This can be addressed by including polycationic carrier molecules in the formulation, which can condense and preserve the RNA while also facilitating its rapid cellular uptake(May,2005).

Conjugate vaccines

Vaccines containing live, attenuated, or inactivated pathogens contain a variety of antigens, both polysaccharide and protein based. However, it's possible that only a limited number of them are needed to elicit protective immunity. The understanding that

each protein has hundreds of potential immunogenic epitopes, not all of which are required has extended this reasoning to proteins (May, 2005). Peptide-based vaccines have sparked interest as a result of this. Antigenic epitopes on a protein, on the other hand, are more than just a sequence of amino acids since the peptides utilized must imitate the immunogenic epitope's shape in the native protein. Computational modeling has proven to be a useful tool for locating and mapping the conformation of immunogenic epitopes within proteins. Because peptide or polysaccharide-based vaccines are less immunogenic than those found on a pathogen's surface, they require the addition of an adjuvant when administered (Sing *et al*.,2021). Another option is to conjugate the antigen to a second 'helper' protein or polysaccharide that has been shown to boost immunogenicity; however, this may cause the immune response to be diverted toward the helper molecule. Approaches to circumvent this difficulty include careful matching and orientation of the target and helper sections of the vaccine, or spatial segregation of the two subunits using carrier systems like as liposomes (Metz*et al*., 2009).

Cellular vaccines

Attempts to employ a similar strategy to vaccinate against cancer have been made due to the history of success of immunization using live attenuated viruses, inactivated viruses, or bacteria. To generate an immune response against specific types of malignancies, attenuated tumor cells have been given. There have been two types of whole cell vaccines used: autologous and allogeneic. Cancers such as lung, colorectal, melanoma, kidney, and prostate cancer have all been studied with autologous cell vaccines. Autologous cell vaccines, on the other hand, are confined to a few types and stages of cancer since they require a significant amount of the patient's tumors for preparation (Sorochi*et al*., 2021). Many whole cell vaccines have been genetically engineered to induce the expression of cytokines, chemokine's, and co-stimulatory molecules in order to boost immune activation. Another type of cellular vaccination makes use of the patient's own immune cells, specifically dendritic cells. Dendritic cell vaccines are made by loading tumor-associated antigens or nuclei into a patient's autologous dendritic cells while they are being treated with immuno-adjuvants. In clinical studies, dendritic cell vaccines have been tested against prostate, melanoma, kidney, and glioma tumors. This vaccine regimen necessitates the collection of the

patient's peripheral blood mononuclear cells, followed by cell culture processing and reinfusion, both of which are time-consuming and costly procedures. While these cell-based techniques are intriguing, they do not appear to contribute to the transition away from live and attenuated vaccines and toward vaccines with lower complexity and production costs that are better suited to treating large populations while decreasing health-care expenditures.(Oyarzún and Kobe, 2016)

Recombinant bacteria as vaccine vectors

Bacteria can be employed as vectors for the in vivo delivery of antigens or DNA, in addition to being widely used to manufacture recombinant subunit vaccines. The low cost and ease of scaling-up production, the availability of well-characterized attenuated strains, the vector's activation of innate immunity, and the efficient delivery to antigen-presenting cells are all potential advantages of this platform. Listeria, Salmonella, Lactococcus, and Bordetella are among the genera being investigated as vaccine vectors. Recombinant bacteria can be utilized as live vaccines, inactivated germs, or even bacterial ghosts with no cytoplasm. In mice, recombinant *Lactococcuslactis* expressing the SARS-coronavirus N protein has been demonstrated to produce antibodies(Wallis *et al*., 2019).

Veterinary Vaccine

Vaccination of animals is used to prevent disease in the animals as well as disease transfer to people. Animals maintained as pets and cattle are both immunized on a regular basis. Wild populations may be vaccinated in some cases. This is often achieved by disseminating vaccine-laced food in a disease-prone region, and it has been used to reduce rabies in raccoons. Rabies vaccination of dogs may be mandated by legislation in areas where rabies is present. Canine distemper, canine parvovirus, infectious canine hepatitis, adenovirus-2, leptospirosis, bordatella, canine Para influenza virus, and Lyme disease are among the various vaccinations available for dogs. Veterinary vaccinations have been administered in people, whether intentionally or accidentally, resulting in certain incidences of disease, most notably brucellosis. However, such occurrences are seldom reported, and little research has been done on the safety and outcomes of such treatments. Human exposure to infections that are not naturally borne in humans, such as Bordetellabronchiseptica, has undoubtedly

risen since the introduction of aerosol immunization in veterinary clinics for veterinary vaccination against a disease can be orders of magnitude less expensive than the human vaccine (Bakarey,2021).

Differentiation of Infected from Vaccinated Animals (DIVA) Vaccines

Differentiation of Infected from Vaccinated Animals (DIVA) vaccines, also known as SIVA (Segregation of Infected from Vaccinated Animals), allow infected and vaccinated animals to be distinguished. The microorganisms prevalent in the field carry at least one epitope less than DIVA vaccines. We can make that distinction with the use of a diagnostic test that detects antibodies against that epitope. Oirschot in 2003 worked in the Central Veterinary Institute in Lelystad (Netherlands) has produced the first DIVA vaccinations (previously known as marker vaccines, and since 1999 known as DIVA vaccines) and companion diagnostic tests. They discovered deletions in the viral genomes of certain current vaccines against pseudorabies (also known as Aujeszky's illness) (among which was the gE gene).

Clinical development

Clinical development is divided into three stages (Fig.2). Small groups of people are given the experimental vaccine during phase I. The clinical research is expanded in phase II, and the vaccine is given to persons who

companion animals in recent years. In certain situations, like as rabies, the

DIVA vaccines and associated diagnostic tests for bovine herpesvirus 1 infections have been developed along the same lines (Koirala *et al*., 2020). The DIVA method has effectively eliminated the pseudorabies virus in a number of nations. Swine populations were heavily vaccinated and monitored using a companion diagnostic test, and diseased pigs were then culled from the herd. Bovine herpesvirus 1 is a virus that infects cattle. In addition, DIVA vaccinations are frequently utilized in clinical practice. Scientists have worked hard to adapt the DIVA principle to a variety of infectious illnesses, including classical swine fever, avian influenza, Actinobacillus pleuropneumonia, and Salmonella infections in pigs, among others (Sing *et al*.,2021; Weniger *et al.*,1999; Wirsiy *et al*.,2021).

have characteristics (such as age and physical health) that are similar to those who will benefit from the new vaccine. Thousands of people are given the vaccine in phase III, and it is examined for efficacy and safety. After a

vaccine is approved and licensed, it is subjected to Phase IV formal, ongoing trials. The Phase I study involves introducing the vaccine candidate to healthy people in order to determine its safety. The Phase I study involves introducing the vaccine candidate to healthy people in order to determine its safety. A Phase I vaccination study consists of healthy volunteers who are given either the candidate vaccine or a "control" treatment, such as a placebo or an adjuvant-containing cocktail, or a proven vaccine (which might be intended to protect against a different pathogen). The major goal of the test is to look for signs of safety (no adverse events) and evidence of an immunological response.(Leroux-Roels *et al*., 2011)

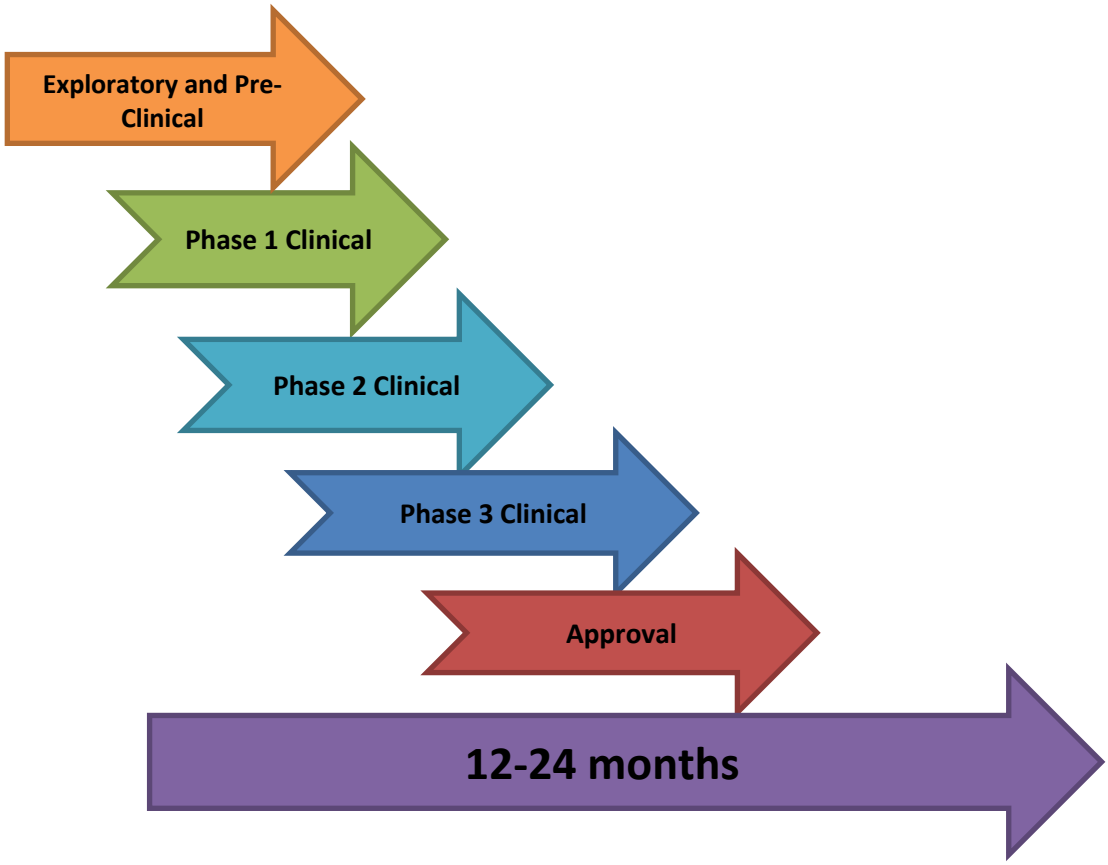

Figure 2. Different phases of vaccine development

Regulatory review and approval

Nearly every stage of vaccine development, manufacture, and marketing clearance involves regulatory difficulties. Regulations apply from the time a vaccine is designed and

clinically tested, through manufacture, and distribution to the general public (May, 2005).

Manufacturing

Vaccine production is a lengthy process. Vaccines take anything from 7 to 36 months to manufacture, package, and deliver to those in need. It entails testing each batch of vaccination at each stage of its trip, as well as repeated quality monitoring of batches by various authorities throughout the world (Lerous-Roels*et al*.,2011)

Quality control

Vaccine quality control used to rely on a range of testing procedures to guarantee that the products were safe and effective. These techniques were created for vaccines whose safety and efficacy were determined after years of research. However, as vaccine manufacturing technology has advanced. Tests can now detect potential risks with a sensitivity that wasn't conceivable just a few years ago, and a growing number of physicochemical approaches allows for considerably improved product characterization. Vaccine regulation includes a number of different measures in addition to sophisticated tests to verify safety. These include supplier audits for characterization of starting materials, cell banking, seed lot systems, adherence to GMP principles, independent release of vaccines on a lot-by-lot basis by national regulatory authorities, and enhanced pre- and post-marketing surveillance for possible adverse events after immunization.(Metz *et al*., 2009)

CONCLUSION AND FUTURE PROSPECTS

Successful vaccine manufacturing necessitates international standardization of starting materials, production and quality control testing, and the establishment of high expectations for regulatory oversight of the entire manufacturing process from start to finish, all while acknowledging that this field is constantly changing. All components, production processes, testing methods, reagents, and standards must adhere to the GMP. Pharmaceutical quality systems, quality assurance techniques and processes, multiple quality controls at each level give guarantee vaccine identity, purity, sterility, efficacy and safety, and suitable infrastructure are all part of these stringent quality criteria (Sorochi *et al*.,2021)

- The vaccine's efficacy or performance is determined by a number of factors, including the disease itself (for some diseases vaccination performs better than for others)
- The vaccination strain (some vaccines are specific to, or at least most effective against, particular strains of the disease)
- Whether the immunization schedule was followed correctly.
- A person's unique reaction to vaccination; some people are "non-responders" to specific vaccines, meaning they do not produce antibodies even after being properly vaccinated.
- Ethnicity, age, or genetic susceptibility, to name a few.

The following are important factors to consider when determining the efficacy of a vaccination programmer:

1. Carefulmodeling to predict the impact of an immunization campaign on disease epidemiology in the medium to long term
2. Ongoing surveillance for the relevant disease following the introduction of a new vaccine
3. Maintaining high immunization rates, even when a disease has become rare.

Vaccines for more than 20 life-threatening diseases are now available, allowing individuals of all ages to enjoy longer, healthier lives. Every year, vaccines prevent 2-4 million deaths from diseases such as diphtheria, tetanus, pertussis, influenza, and measles. Immunization is an indisputable human right and an important component of primary health care. It's also one of the most cost-effective health investments available. Vaccines are also important for preventing and controlling infectious disease outbreaks. They are essential in the fight against antimicrobial resistance and support global health security (Wirsiy *et al.,*2021). Despite significant advances, far too many people around the world – including approximately 20 million infants each year – lack adequate immunization access. Progress has slowed or even reversed in some nations, and there is a serious danger that complacency will destroy previous successes (Wallis *et al*.,2019)

Vaccines contain pure materials obtained from dead or inactivated organisms. Vaccines come in a variety of shapes and sizes. These are many approaches of reducing

the risk of sickness while maintaining the ability to elicit a positive immunological response. Vaccines can be monovalent (also known as univalent) or multivalent (also known as multivalent) (also called polyvalent). A monovalent vaccine is intended to protect against a single antigen or microbe. A multivalent or polyvalent vaccine protects against two or more strains of the same microbe, or two or more germs altogether. A Greek or Latin prefix might be used to indicate the valiancy of a multivalent vaccine (e.g., tetravalent or quadrivalent). A monovalent vaccine may be beneficial in some situations for eliciting a high immune response quickly. When two or more vaccines are combined in the same formulation, they will create problems (Sing *et al*.,2021) This is particularly common with live attenuated vaccinations, in which one of the vaccine components is stronger than the others, suppressing the growth and immune response to the others. This behavior was originally observed with the trivalent Sabin polio vaccine, where the amount of serotype 2 viruses in the vaccine had to be lowered to avoid interfering with the "take" of the serotype 1 and 3 viruses. This behavior has also been discovered to be a problem with current dengue vaccines in which the DEN-3 serotype predominates and suppresses the response to the DEN-1, 2, and 4 serotypes. When it's time for a booster, people who have had a bad reaction to adsorbed tetanus toxoid may be given the basic vaccine.(Yang *et al*., 2016)

Preservatives may be added to vaccines to avoid contamination by bacteria or fungi. Preservatives may be utilized at many phases of vaccine manufacture, and the most advanced measurement methods may identify residues of them in the completed product, just as they may in the environment.

The following excipients and leftover manufacturing chemicals are present or may be present in vaccine formulations, in addition to the active vaccine:

- Adjuvants such as aluminum salts or gels are used.
- Adjuvants are added to vaccines to stimulate a faster, more powerful, and longer-lasting immune response, allowing for a lower vaccination dose.
- Antibiotics are used in certain vaccinations to prevent bacteria from growing during manufacturing and storage.

- Because influenza and yellow fever vaccinations are made from chicken eggs, they include egg protein. Other proteins might be present as well.
- Toxoid vaccinations utilize formaldehyde to inactivate bacterial products.
- In a few vaccines, stabilizers such as monosodium glutamate (MSG) and 2-phenoxyethanol are used to keep the vaccine stable whether it is exposed to heat, light, acidity, or humidity.
- Thiomersal is a mercury-based antibacterial that is added to vaccination vials containing several doses to avoid contamination and the development of potentially dangerous microorganisms.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest for publication of this review.

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CHARACTERIZATION OF *CURVULARIA LUNATA;* FOLIAR FUNGAL PATHOGEN OF ORNAMENTAL PALMS

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ABSTRACT

Agriculture is the backbone of any economy and an essential field of study considered as a challenge for many researchers today. Fungal diseases are a constant threat to agricultural commodities resulting huge decline in quality and quantity. The early detection and classification of fungal plant diseases are crucial for preventing growing diseases and hence yield reduction. In the present study, leaf spot on ornamental palm, caused by *Curvularia lunata*, was identified as a major fungal pathogen declining the quality of palm trees. The fungus was isolated from lesions on leaves, and pathogenicity was confirmed. Pathogenicity assay confirmed that *C. lunata* is a causal agent of leaf spot of ornamental palms. The fungus was identified based on morphological and microscopic characteristics. One of the characteristics of palm disease is that the disease symptoms mostly appear as spotted on the leaves at its nursery stage. So, the need of time is to develop a novel and authentic disease detection system that may detect and separate the infected palm trees earlier to prevent the reduction of palm yields.

Keywords; *Curvularia lunata*, Ornamental Palm Tree, Pathogenicity Assay.

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INTRODUCTION

Palms include a natural and characteristic, yet remarkably tropical plant comprising about 2500 species in 184 genera and is best and famous in, the Central South America

western Pacific, Australia tropical Asia, and Madagascar (Dransfield et al., 2008). Numerous palms hold great worldwide commercial significance. The date palm and coconut palm are considered two of the world's ten most significant agronomic crops (Janick & Paull, 2008). Palms are good source of food and oil, fiber for coir, string, bins, caps and carpets; cane used in furniture manufacture, tannin, timber, straw for roofing material, wine and other drinks (Howard et al., 2001). Currently there is bigger command for date palm fruits in all over the world. To full fill this mandate numerous production techniques have been exploited among them micro propagation which has been used in Iraq and various other countries for large-scale multiplication of date palm. Palm a tropical tree crop, is important for the industrial production of vegetable oil with Indonesia and Malaysia and makes up the highest plantation area in Southeast Asia (Carter et al., 2007).Quality of palm tree is mainly affected by various fungal rots. Pink rot (*Nalanthamala vermoesenii*), false smut (*Graphiola phoenicis*) and black rot (*Ceratocystis paradoxa*) are the most destructive fungal diseases of palm, while *Botrytis phaeriaceae*, Pestalotiopsis spp., and Phomopsis spp., are found to be the common diseases (Cabrera et al., 1990). Many of the leaf spots and leaf blights of palm are initiated by fungal pathogens. Generally, the symptoms appearance alike which fungus is causing the spot or blight. Palm leaflets are normally formed in cross section with the middle vein at the apex of the v shape (Broschat, 2016). Armengol et al., (2002) reported that Some species of Phytophthora cause main diseases of palms all over the world with bud (heart) rot the very common and disturbing disease. A lethal bud rot of numerous species of palms is caused by Phytophthora palmivora (Elliott et al., 2004).

Curvularia leaf spot disease caused by *Curvularia lunata* is a unique fungus in appearance and life cycle, but it is broadly dispersed all over the date palm-growing domain. Although several palm species have been recognized as hosts of this fungus the disease is widespread worldwide (Elliott et al., 2010). To evaluate the cause of decline in palm quality the present study was focused on identification and characterization of foliar fungal rot of ornamental palm trees.

MATERIAL AND METHODS

The current study was designed to determine the isolation and preservation of important foliar fungal pathogens along with their morphological and cultural studies. An extensive survey was conducted of district Islamabad Pakistan (33.6841° N, 73.0480° E) during year 2021 to identify foliar fungal rot of ornamental palm trees. Infected leaves were washed thrice with distilled water and blotted dry. Diseased portions were excised into $5mm^3$ segments including diseased as well as healthy portion. These segments were surface disinfected with one percent sodium hypochlorite (NaOH) for about 1-2mins and washed twice with sterilized distilled water. Disinfected samples were blotted dry using sterilized filter papers and were placed on PDA media petri plates and incubated at 25°C±2 for a week. Isolated fungal pathogen was purified by single spore method and hyphal tip method. After purification fungi was identified under microscope based on colony color, colony texture, mycelia appearance, hyphal septation. Spore size and shape was observed under 100X lens of Nikon YS100 microscope using fungal taxonomic identification keys.

Pathogenicity Assay was conducted on ornamental palms for the confirmation of Koch's postulates. The inoculum of each strain was prepared by flooding the agar surface with 10 mL of sterile distilled water (SDW) and scraping with a spatula. The resulting spore suspension was filtered through four layers of cheesecloth and the filtrate was diluted with sterile distilled water whereas the conidial concentration was adjusted to 10^6 conidia mL^{-1} using a haemocytometer. One-year healthy ornamental palm leaves were sterilized with 0.5% sodium hypochlorite (NAOH) solution and placed in sterilized boxes. Further inoculation was done by spraying inoculum on with approximately 50 mL of spore suspension. Another healthy ornamental palm leaves inoculated for controls was sprayed with sterile distilled water and incubated for 5- 10 days till the appearance of symptoms.

RESULTS AND DISCUSSION

Disease incidence of four abundantly found palm spp., was recorded in Islamabad Pakistan. Maximum disease incidence was recorded 74.21% of Italian palm and minimum disease incidence recorded was 43.11% of table palm (Figure 1). A total of

23 isolates were subjected to morphological studies. The pathogen identified on morphological and microscopic features was *Curvularia lunata* initiating black leaf spot of bamboo palm. Colony color varied from light brown to brownish black and light brown color. Among 23 isolates, CURL3 was observed with maximum colony diameter and CURL2 was recorded showing minimum colony diameter 66 mm. Colony margin color varied from blackish brown to dark black with somewhat straw color appearance. Growth pattern of colonies was recorded suede-downy, irregular to appressed having fluffy growth. Underside of petri dish was observed blackish-brown. Conidia were boat shaped showing olive green to dark brown (Figure 2). Spore shape of isolates was straight to curved and maximum spore length 38.14±4.34 µm was observed in CURL3 whereas minimum spore length 22.11±2.13 µm was observed in CURL5 (Table 1; Figure 3). During pathogenicity assay 06 isolates of *Curvularia lunata* were found to be highly pathogenic. Fungal isolates were preserved in 15% glycerol solution and were placed in -20 C for long term usage.

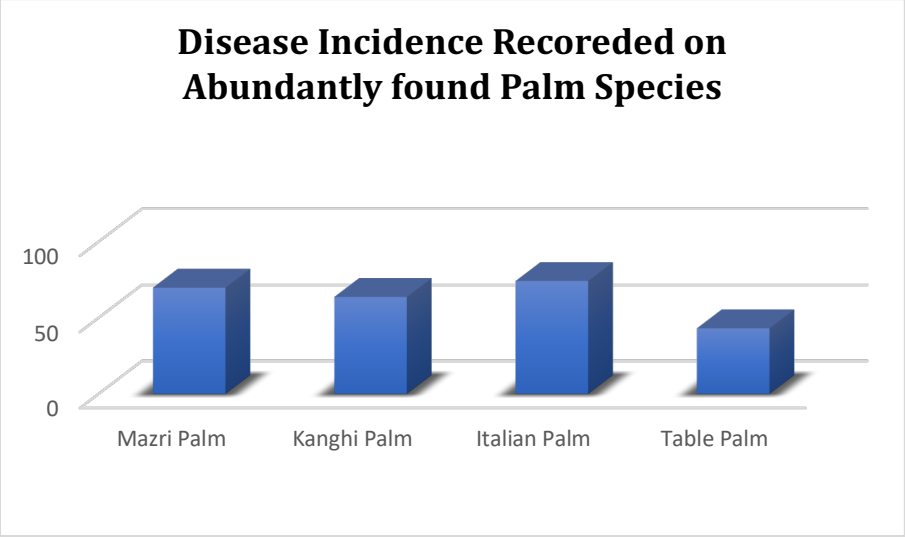

Figure 1 Disease Incidence on Abundantly found Palm Species

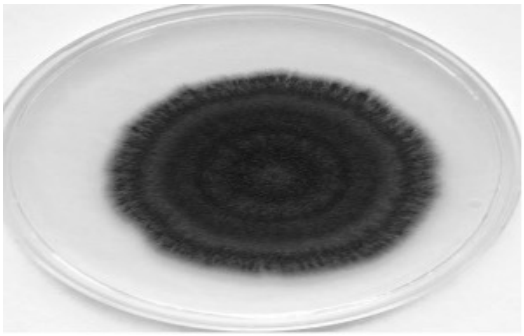

Figure 2 *Curvularia lunata* pure culture on PDA media

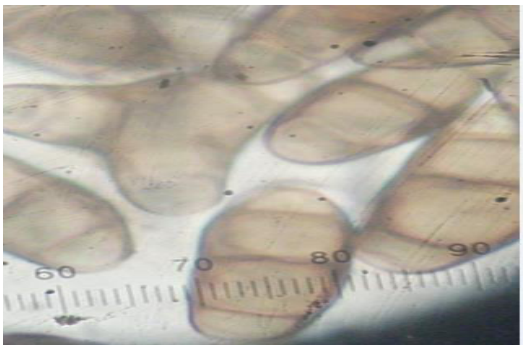

Figure 3 Spores observed under Nikon Microscope

Table 1: Cultural characterization of *Curvularia lanata*

Isolate ID	Colony Color	Conidia Shape	Colony Growth Pattern	Colony Diameter	Spore size (Length)
CURL1	Light black-brown	Boat Shaped	Suede-downy	68 mm	31.27±3.14 µm
CURL2	Blackish-brown	Enlarged	Irregular appressed	66 mm	33.41±4.11 µm
CURL3	Black	Boat Shaped	Fan-like appearance	72 mm	38.14±4.34 µm
CURL4	Light brown	Boat Shaped	Smooth	67 mm	29.25±3.21 µm
CURL5	Dark Black	Boat Shaped	Suede-downy	69 mm	22.11±2.13 µm
CURL6	Black	Boat Shaped	Suede-downy	71 mm	29.91±2.17 µm

In another study reported by Manamgoda et al., (2012) morphological features of fungal pathogen causing leaf spot of ornamental palm were evaluated where colonies were observed whitish to greyish black mycelial growth after 8 days of incubation at 26 °C, moreover colonies were blackish, conidiophores were septate with dark brown scars, unbranched, and fexuose at apical region, conidia were dark brown, boat shaped with rounded tips, smooth walled, in sympodial order and 25–33×12–14 µm size (Manamgoda et al. 2012). It is pertinent to mention that *Penicillium* spp., *Fusarium* spp., *Curvularia* spp., *Aspergillus* spp., and *Alternaria* spp., are important pathogens of ornamental palms causing significant yield losses to date-palm (Bokhary, 2010; Khudhair et al., 2015; Suwannarach et al., 2015; Polizzi and Vitale, 2003). In addition, *Curvularia lunata*, *C. maculans* and *Helminthosporium halodes* have also been reported as oil palm leaf spot pathogens (National Research Council 1993). Furthermore, leaf spot on ornamental and oil palms is initiated by *Curvularia lunata* in Pakistan and worldwide (Farr and Rossman 2020).

CONFLICT OF INTEREST

No conflict of interest declared by authors.

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EFFECT OF FOLIAR APPLICATION OF ZINC ON YIELD AND QUALITY OF *ALLIUM SATIVUM* VAR. OPHIOSCORODON

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ABSTRACT

The impact of individual micronutrients on the production course processes and their impact on the quality of *Allium sativum* is an important concern in horticultural sciences. In the present study it was revealed that fertilization with microelements in the minimum and optimal norms contributed to a significant increase in yield, the application of maximum norms led to a decrease in productivity. Our results revealed that all four applied treatments of Zinc foliar application on garlic exhibited difference in plant height, stem diameter, leaf length and number of leaves per plant. The effect of foliar zinc treatments applied were observed in the form of bulbs germination after 90, 100, 110 and 120 days according to the variant and storage regime. Further research is to study the combinations of the studied micronutrient on physiological processes and biochemical parameters to optimize their norms for local fertilization.

Keywords: *Allium sativum*, Zinc, Foliar Application

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INTRODUCTION

Garlic is an important vegetable crop of Allaceae family in bulbous group that was originated from central Asia and later spread to Mediterranean region ((Mahala et al.,

2022; Simon, 2001; Kigori, 2001). Garlic is commonly used as a spice or in the medicinal purposes and is generally cultivated for both local and export purposes. The bulb of garlic holds numerous natural compounds, bulblets, so called as cloves and these cloves are different in size surrounded by layers of white scale leaves. Allicin is the main biological active component of freshly crushed garlic cloves, which is produced by the degradation of Allin, from results of alliinase activity (Bocchini, et al., 2001). Micronutrients play an active role in the plant metabolic process from cell wall development to respiration, photosynthesis, chlorophyll formation, enzymes activity, nitrogen fixation etc. Micronutrients work as a co-enzyme for many enzymes (Lawrence et al., 2011; Ameri et al., 2012).

The use of micronutrients in soil nutrition is the pillars of agriculture in developed proper plant nutrition are one of the most important factors in improving the quality and quantity of plants product. Zinc is required in small but critical concentrations to allow several key plant physiological pathways to function normally (Alloway, 2002; Mousavi et al., 2011; Yousuf et al., 2016). By utilizing of fertilizers contain zinc and other micronutrients, performance on quality of crops is increasing and with shortage of these elements due to declines in plant photosynthesis and destroyed RNA, amount of solution carbohydrates and synthesis of protein decreased and then performance and quality of crop will be decreased (Mousavi et al., 2007).

Zinc (Zn) is a vital mineral element for plant development and holds great beneficial impact on various plant growth aspects. It is involved in several biochemical processes, for instance, the synthesis of proteins, chlorophyll, enzymes, and metabolic turnover (Alloway et al., 2008). However, the deficiency of Zn has negative consequences on plants; therefore, utilizing Zn oxide nano-fertilizer (ZNF) as a ZnO NPs is an intriguing concept that is presently being investigated (Choudhary et al., 2014). For instance, positive effects of the application of nano-ZnO were reported on seed germination, seedling vigour, leaf chlorophyll content, stem and root growth in peanut (Yadav et al., 2018), and the positive effects of nano-ZnO contrasted with the negative effects on vegetable seed germination of a bulk form of ZnO (Manna, 2013).

The effects of applied micronutrient as a foliar spraying on growth and yield of garlic plants Mondal et al. (2016) and Yousuf

et al. (2016) recorded that the foliar application with micronutrient improved the vegetative growth enhanced yield and yield attributes of garlic (Chanchan et al., 2015). In this respect, application of zinc and boron has positive effects on plant growth and improvement of production of garlic. In addition, zinc application improves roots system which results in better absorption of water and other dissolved nutrients and consequently improves different organs and entire plant growth. Moreover, roots are unable to absorb some important nutrients such as zinc, Because of soil properties, such as high pH and carbonate. Thus, in this situation, foliar Praying is better as compared to soil application (Mondal et al., 2011).

Foliar fertilizers are dilute fertilizer solutions applied directly to plant leaves. As with soil application of fertilizer, the goal of foliar fertilization is to supply plants with the nutrients needed for good growth. It is a technique of feeding plants by applying liquid fertilizer directly to their leaves. Plants are able to absorb essential elements through their leaves. The absorption takes place through their stomata and also through their epidermis. It is the application of fertilizers to foliage of the crop as spray solution is known as foliar spray. This method is suitable for application of small quantities of fertilizers, especially micronutrients.

Zinc is one of the seven micronutrients vital for the crop growth. Zinc plays a considerable role in various enzymatic and physiological activities and performs many catalytic functions in plant system besides alteration of carbohydrates, chlorophyll and protein synthesis (Pramanik and Tripathy, 2017). Foliar application of zinc improves morpho-physiological and antioxidant defense mechanisms and agronomic grain biofortification of wheat (*Triticum aestivum* L.) under water stress. Foliar application of zinc (Zn) to crops is an effective way to increase the grain concentration of Zn. However, the development of more efficient foliar Zn fertilizers is limited by a lack of knowledge regarding the distribution, mobility, and speciation of Zn in leaves once it is taken up by the plant (El-Sayed et al., 2015).

The aim of present study was to determine the optimal microelement (Zn) norms for the garlic to qualify the impact of different concentrations of Zinc on the growth, physiological processes and yield of garlic.

MATERIAL AND METHODOLOGY

The experiment was conducted at the experimental field of Vegetable Crops Research Program, Horticultural Research Institute, National Agricultural Research Centre (NARC) Islamabad during 2022-23. Garlic cloves were sown in pots under plastic tunnel. The experiment conducted under tunnel. Three treatments were selected for Zinc foliar sprays viz; T1 (0.1%), T2 (0.2%), T3 (0.3%) and readings were taken after four different intervals. Four different plant parameters were recorded after Zn application viz: Plant height (cm), Stem diameter (mm), Leaf length (cm) and number of leaves per plant^{-1}. The data analysis was performed by analysis of variance (ANOVA) and means were separated using Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Our results revealed that all four applied treatments of Zn foliar application on garlic exhibited difference in plant height, stem diameter, leaf length and number of leaves per plant, respectively. Whereas it is pertinent to mention that highest plant height was recorded 26.67 in T3 after 120 DAS and lowest was recorded 21.67% in T2 (Table 1). Maximum stem diameter was measured 1.49 in T1 after 110 DAS and minimum was recorded 1.29 after 90 DAS (Table 2). Leaf length and number of leaves per plant after applying all treatments were recorded (Table 3; Table 4).

Table 1: Comparison of plant height under different foliar applications of Zn

Treatments	90 DAS	100 DAS	110 DAS	120 DAS
CK: Control	22.10 b	24.07 b	24.93 b	27.47 b
T_1: 0.1 % Zn foliar spray	24.73 a	25.67 a	30.60 a	31.27 a
T_2: 0.2 % Zn foliar spray	21.67 c	22.27 c	25.93 b	26.47 c
T_3: 0.3 % Zn foliar spray	22.27 b	22.73 c	25.60 b	26.67 c

DAS: days after sowing

Table 2: Comparison of stem diameter under different foliar applications of Zn

Treatments	90 DAS	100 DAS	110 DAS	120 DAS
CK: Control	1.31 a	1.34 a	1.43 a	1.49 a
T_1: 0.1 % Zn foliar spray	1.41 a	1.47 a	1.49 a	1.55 a

T_2: 0.2 % Zn foliar spray	1.37 a	1.39 a	1.43 a	1.46 a
T_3: 0.3 % Zn foliar spray	1.29 a	1.36 a	1.39 a	1.47 a

DAS: days after sowing

Table 3: Comparison of leaf length under different foliar applications of Zn

Treatments	90 DAS	100 DAS	110 DAS	120 DAS
CK: Control	18.00 b	20.27 b	21.80 c	22.80 d
T_1: 0.1 % Zn foliar spray	20.67 a	21.53 a	26.00 a	27.20 b
T_2: 0.2 % Zn foliar spray	17.93 c	18.67 c	22.60 b	23.40 c
T_3: 0.3 % Zn foliar spray	18.53 b	20.40 b	22.53 b	28.00 a

DAS: days after sowing

Table 4: Comparison of number of leaves per plant under foliar applications of Zn

Treatments	90 DAS	100 DAS	110 DAS	120 DAS
CK: Control	5.27 a	5.27 a	5.47 a	5.47 a
T_1: 0.1 % Zn foliar spray	4.93 b	5.13 a	5.53 a	5.80 a
T_2: 0.2 % Zn foliar spray	4.87 b	5.07 a	5.47 a	5.60 a
T_3: 0.3 % Zn foliar spray	4.87 b	5.07 a	5.27 a	5.53 a

DAS: days after sowing

DISCUSSION

Our results are in similarity with reported work of Chanchan et al., (2013), where Zn applied as foliar application enhanced garlic yield. Application of zinc (Zn) can reduce Cadmium uptake by plants, as both these metals are generally antagonistic in soil–plant systems (Wang et al., 2018). Similarly, in another study yield parameters and storage quality of garlic (*Allium sativum* L.) var. G-282 were enhanced after Zn application (Yadav et al., 2018).

In another study, application of Zn significantly increased the bulb weight (73.9 g), bulb yield (45 t/ha) when applied with 2, 4-D (3 ppm) as foliar spray. Zinc application significantly influenced the bulb quality and recorded the highest a grade bulbs and the lowest poor quality C grade bulbs when

applied with 2, 4-D (81.9%) as foliar spray (Manna et al., 2016). Arif et al. in (2006) reported that foliar application can guarantee the availability of nutrients to crops for obtaining higher yield. Srivastava et al. (2005) reported that boric acid at 0.1% and zinc sulfate at 0.4% resulted in maximum bulb yield and total soluble solids. The application of micronutrients soil or foliar spray significantly influenced bulb yield of onion crop (Pramanik and Tripathy, 2017; Singh et al., 2015).

CONCLUSION

It is pertinent to mention that balanced application of zinc holds highest influence on *Allium sativum* growth various physiological parameters. After the zinc application at maximum norm a significant positive impact was observed on plant height, stem diameter, leaf length and number of leaves per plant. The most significant increase in bulb weight was influenced by all applied foliar treatments of zinc at different days interval. The use of zinc contributed to higher accumulation and extended the marketability of garlic bulbs.

CONFLICT OF INTEREST

No potential Conflict of Interest is declared.

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APPLICATION OF BIOMASS FOR BIO BASED INNOVATIVE INDUSTRIAL PRODUCTS

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ABSTRACT

Biomass is renewable organic material that comes from plants and animals. Biomass includes wood, agricultural wastes (crop residues) and cow-dung. Biomass is another form in which solar energy manifests itself. This is because all the plants and trees which provide biomass (like wood) used sun's energy to grow. Biomass is material that comes from living things. Because living things have energy, biomass provides a renewable source of energy to fuel our vehicles, heat our homes, and make electricity. Biomass energy is generated by living beings. The most common biomass materials used for energy include plants viz, corn, soy, etc. The energy from these organisms can be burned to create heat or converted into electricity.

Keywords; Biomass, Bioenergy, economic importance

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INTRODUCTION

Biomass is organic, meaning it is made of material that comes from living organisms, such as plants and animals. The most common biomass materials used for energy are plants, wood, and waste. These are called biomass feedstocks. Biomass energy can also be a non-renewable energy source. Renewable energy is energy that is collected

from renewable resources There are five major renewable energy sources

- Solar energy from the sun.
- Geothermal energy from heat inside the earth.
- Wind energy.
- Biomass from plants.
- Hydropower from flowing water.

First-generation biofuels include ethanol and biodiesel and are directly related to a biomass that is more than often edible. Ethanol is generally produced from the fermentation of C_6 sugars (mostly glucose) using classical or GMO yeast strains such as Saccharomyces cerevisiae

Second-generation biofuels, also known as advanced biofuels, are fuels that can be manufactured from various types of non-food biomass. Biomass in this context means plant materials and animal waste used especially as a source of fuel.

The third-generation bioethanol is focused on the use of marine organisms such as algae. The public acceptance on the ability of algae to provide biomass for bioethanol production is positive as this action can limit the feedstock competition from agriculture plants. The fourth-generation biofuels combine genetically engineered feedstock with genomically synthesized microorganisms, such as cyanobacteria, to efficiently generate bioenergy, and they are made using nonarable land similar to third-generation biofuels.

MATERIAL AND METHODS

As biomass is an important industrial products, therefore different information were collected and emerged into one single documents that will provide prompt knowledge to research workers

Biomass—renewable energy from plants and animals

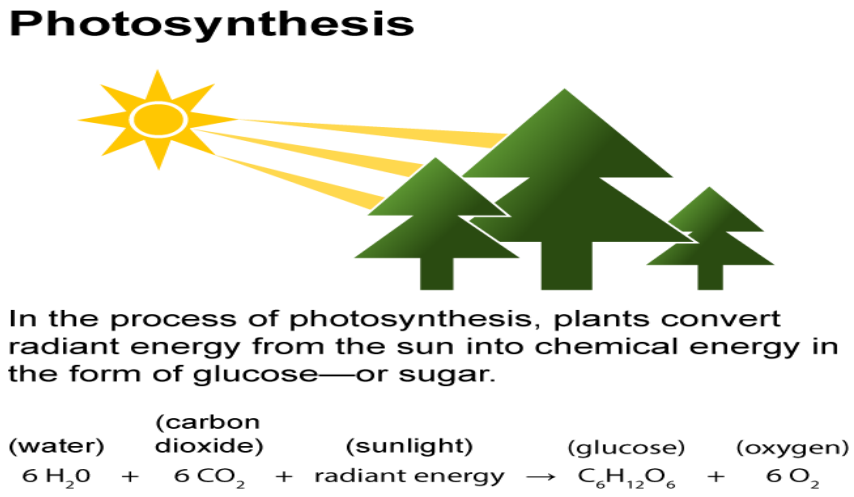
Biomass is renewable organic material that comes from plants and animals. Biomass was the largest source of total annual U.S. energy consumption until the mid-1800s. Biomass

continues to be an important fuel in many countries, especially for cooking and heating in developing countries. The use of biomass fuels for transportation and for electricity generation is increasing in many developed countries as a means of avoiding carbon dioxide emissions from fossil fuel use. In 2020, biomass provided nearly 5 quadrillion British thermal units (Btu) and about 5% of total primary energy use in the United States.

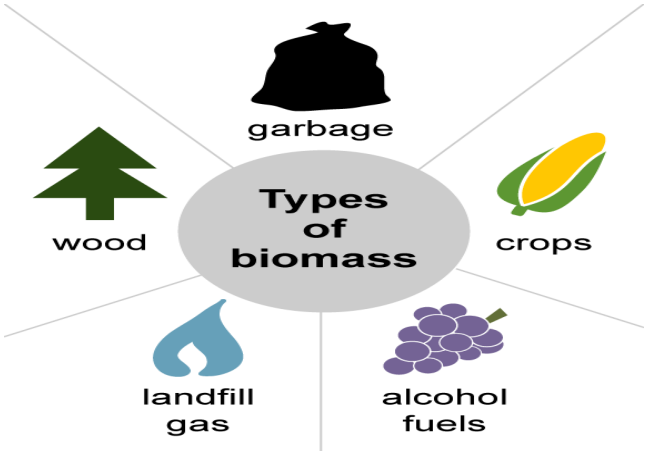
Biomass contains stored chemical energy from the sun. Plants produce biomass through photosynthesis. Biomass can be burned directly for heat or converted to renewable liquid and gaseous fuels through various processes.

Biomass sources for energy include:

- Wood and wood processing wastes—firewood, wood pellets, and wood chips, lumber and furniture mill sawdust and waste, and black liquor from pulp and paper mills
- Agricultural crops and waste materials—corn, soybeans, sugar cane, switchgrass, woody plants, and algae, and crop and food processing residues
- Biogenic materials in municipal solid waste—paper, cotton, and wool products, and food, yard, and wood wastes
- Animal manure and human sewage



Source: Adapted from The National Energy Education Project (public domain)



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Converting biomass to energy

Biomass is converted to energy through various processes, including:

- Direct combustion (burning) to produce heat
- Thermochemical conversion to produce solid, gaseous, and liquid fuels
- Chemical conversion to produce liquid fuels
- Biological conversion to produce liquid and gaseous fuels

Direct combustion is the most common method for converting biomass to useful energy. All biomass can be burned directly for heating buildings and water, for industrial process heat, and for generating electricity in steam turbines.

Thermochemical conversion of biomass includes *pyrolysis* and *gasification*. Both are thermal decomposition processes in which biomass feedstock materials are heated in closed, pressurized vessels called *gassifiers* at high temperatures. They mainly differ in the process temperatures and amount of oxygen present during the conversion process.

- Pyrolysis entails heating organic materials to 800–900°F (400–500 °C) in the near complete absence of free oxygen. Biomass pyrolysis produces fuels such as charcoal, bio-oil, renewable diesel, methane, and hydrogen.
- Hydrotreating is used to process bio-oil (produced by *fast pyrolysis*) with

hydrogen under elevated temperatures and pressures in the presence of a catalyst to produce renewable diesel, renewable gasoline, and renewable jet fuel.

- Gasification entails heating organic materials to 1,400–1700°F (800–900°C) with injections of controlled amounts of free oxygen and/or steam into the vessel to produce a carbon monoxide and hydrogen rich gas called synthesis gas or *syngas*. Syngas can be used as a fuel for diesel engines, for heating, and for generating electricity in gas turbines. It can also be treated to separate the hydrogen from the gas, and the hydrogen can be burned or used in fuel cells. The syngas can be further processed to produce liquid fuels using the Fischer–Tropsch process.

A chemical conversion process known as *transesterification* is used for converting vegetable oils, animal fats, and greases into fatty acid methyl esters (FAME), which are used to produce biodiesel.

Biological conversion includes fermentation to convert biomass into ethanol and anaerobic digestion to produce renewable natural gas. Ethanol is used as a vehicle fuel. Renewable natural gas—also called *biogas* or *biomethane*—is produced in anaerobic digesters at sewage treatment plants and at dairy and livestock operations. It also forms in and may be captured from solid waste landfills. Properly treated renewable natural gas has the same uses as fossil fuel natural gas.

Researchers are working on ways to improve these methods and to develop other ways to convert and use more biomass for energy.

How much biomass is used for energy?

In 2020, biomass provided about 4,532 trillion British thermal units (TBtu), or about 4.5 quadrillion Btu and equal to about 4.9% of total U.S. primary energy consumption. Of that amount, about 2,101 TBtu were from wood and wood-derived biomass, 2,000 TBtu were from biofuels (mainly ethanol), and 430 TBtu were from the biomass in municipal wastes.Biomass contains energy first derived from the sun: Plants absorb the sun's energy through photosynthesis, and convert carbon dioxide and water into nutrients (carbohydrates).

The energy from these organisms can be

transformed into usable energy through direct and indirect means. Biomass can be burned to create heat (direct), converted into electricity (direct), or processed into biofuel (indirect).

RESULTS AND DISCUSSION

Thermal Conversion Biomass can be burned by thermal conversion and used for energy. Thermal conversion involves heating the biomass feedstock in order to burn, dehydrate, or stabilize it. The most familiar biomass feedstocks for thermal conversion are raw materials such as municipal solid waste (MSW) and scraps from paper or lumber mills.

Different types of energy are created through direct firing, co-firing, pyrolysis, gasification, and anaerobic decomposition.

Before biomass can be burned, however, it must be dried. This chemical process is called torrefaction. During torrefaction, biomass is heated to about 200° to 320° Celsius (390° to 610° Fahrenheit). The biomass dries out so completely that it loses the ability to absorb moisture, or rot. It loses about 20% of its original mass, but retains 90% of its energy. The lost energy and mass can be used to fuel the torrefaction process.

During torrefaction, biomass becomes a dry, blackened material. It is then compressed into briquettes. Biomass briquettes are very hydrophobic, meaning they repel water. This makes it possible to store them in moist areas. The briquettes have high energy density and are easy to burn during direct or co-firing.

Direct Firing and Co-Firing Most briquettes are burned directly. The steam produced during the firing process powers a turbine, which turns a generator and produces electricity. This electricity can be used for manufacturing or to heat buildings.

Biomass can also be co-fired, or burned with a fossil fuel. Biomass is most often co-fired in coal plants. Co-firing eliminates the need for new factories for processing biomass. Co-firing also eases the demand for coal. This reduces the amount of carbon dioxide and other greenhouse gases released by burning fossil fuels.

Pyrolysis

Pyrolysis is a related method of heating biomass. During pyrolysis, biomass is heated to 200° to 300° C (390° to 570° F) without the presence of oxygen. This keeps it from combusting and causes the biomass to be chemically altered.

Pyrolysis produces a dark liquid called pyrolysis oil, a synthetic gas called syngas, and a solid residue called biochar. All of these components can be used for energy.

Pyrolysis oil, sometimes called bio-oil or biocrude, is a type of tar. It can be combusted to generate electricity and is also used as a component in other fuels and plastics. Scientists and engineers are studying pyrolysis oil as a possible alternative to petroleum.

Syngas can be converted into fuel (such as synthetic natural gas). It can also be converted into methane and used as a replacement for natural gas.

Biochar is a type of charcoal. Biochar is a carbon-rich solid that is particularly useful in agriculture. Biochar enriches soil and prevents it from leaching pesticides and other nutrients into runoff. Biochar is also an excellent carbon sink. Carbon sinks are reservoirs for carbon-containing chemicals, including greenhouse gases.

Gasification

Biomass can also be directly converted to energy through gasification. During the gasification process, a biomass feedstock (usually MSW) is heated to more than 700° C (1,300° F) with a controlled amount of oxygen. The molecules break down, and produce syngas and slag.

Syngas is a combination of hydrogen and carbon monoxide. During gasification, syngas is cleaned of sulfur, particulates, mercury, and other pollutants. The clean syngas can be combusted for heat or electricity, or processed into transportation biofuels, chemicals, and fertilizers.

Slag forms as a glassy, molten liquid. It can be used to make shingles, cement, or asphalt.

Industrial gasification plants are being built

all over the world. Asia and Australia are constructing and operating the most plants, although one of the largest gasification plants in the world is currently under construction in Stockton-on-Tees, England. This plant will eventually be able to convert more than 350,000 tons of MSW into enough energy to power 50,000 homes.

Anaerobic Decomposition

Anaerobic decomposition is the process where microorganisms, usually bacteria, break down material in the absence of oxygen. Anaerobic decomposition is an important process in landfills, where biomass is crushed and compressed, creating an anaerobic (or oxygen-poor) environment.

In an anaerobic environment, biomass decays and produces methane, which is a valuable energy source. This methane can replace fossil fuels.

In addition to landfills, anaerobic decomposition can also be implemented on ranches and livestock farms. Manure and other animal waste can be converted to sustainably meet the energy needs of the farm.

Biofuel

Biomass is the only renewable energy source that can be converted into liquid biofuels such as ethanol and biodiesel. Biofuel is used to power vehicles and is being produced by gasification in countries such as Sweden, Austria, and the United States.

Biofuels do not operate as efficiently as gasoline. However, they can be blended with gasoline to efficiently power vehicles and machinery, and do not release the emissions associated with fossil fuels.

Ethanol is made by fermenting biomass that is high in carbohydrates, such as sugar cane, wheat, or corn. Biodiesel is made from combining ethanol with animal fat, recycled cooking fat, or vegetable oil.

Ethanol requires acres of farmland to grow bio crops (usually corn). About 1,515 liters (400 gallons) of ethanol is produced by an acre of corn. But this acreage is then unavailable for growing crops for food or

other uses. Growing enough corn for ethanol also creates a strain on the environment because of the lack of variation in planting, and the high use of pesticides.

Ethanol has become a popular substitute for wood in residential fireplaces. When it is burned, it gives off heat in the form of flames, and water vapor instead of smoke.

Biochar

Biochar, produced during pyrolysis, is valuable in agricultural and environmental use. When biomass rots or burns (naturally or by human activity), it releases high amounts of methane and carbon dioxide into the atmosphere. However, when biomass is charred, it sequesters, or stores, its carbon content. When biochar is added back to the soil, it can continue to absorb carbon and form large underground stores of sequestered carbon—carbon sinks—that can lead to negative carbon emissions and healthier soil.

Biochar also helps enrich the soil. It is porous. When added back to the soil, biochar absorbs and retains water and nutrients.

Biochar is used in Brazil's Amazon rain forest in a process called slash-and-char. Slash-and-char agriculture replaces slash-and-burn, which temporarily increases the soil nutrients but causes it to lose 97% of its carbon content. During slash-and-char, the charred plants (biochar) are returned to the soil, and the soil retains 50% of its carbon. This enhances the soil and leads to significantly higher plant growth.

BlackLiquor

When wood is processed into paper, it produces a high-energy, toxic substance called black liquor. Until the 1930s, black liquor from paper mills was considered a waste product and dumped into nearby water sources.

However, black liquor retains more than 50% of the wood's biomass energy. With the

invention of the recovery boiler in the 1930s, black liquor could be recycled and used to power the mill. In the U.S., paper mills use nearly all their black liquor to run their mills, and the forest industry is one of the most energy-efficient in the nation as a result.

More recently, Sweden has experimented in gasifying black liquor to produce syngas, which can then be used to generate electricity.

Hydrogen Fuel Cells

Biomass is rich in hydrogen, which can be chemically extracted and used to generate power and to fuel vehicles. Stationary fuel cells are used to generate electricity in remote locations, such as spacecraft and wilderness areas. Yosemite National Park in the U.S. state of California, for example, uses hydrogen fuel cells to provide electricity and hot water to its administration building. Hydrogen fuel cells may hold even more potential as an alternative energy source for vehicles. The U.S. Department of Energy estimates that biomass has the potential to produce 40 million tons of hydrogen per year. This would be enough to fuel 150 million vehicles.

Currently, hydrogen fuel cells are used to power buses, forklifts, boats, and submarines, and are being tested on airplanes and other vehicles.

However, there is a debate as to whether this technology will become sustainable or economically possible. The energy that it takes to isolate, compress, package, and transport the hydrogen does not leave a high quantity of energy for practical use.

Biomass and the Environment

Biomass is an integral part of Earth's carbon cycle. The carbon cycle is the process by which carbon is exchanged between all layers of the Earth: atmosphere, hydrosphere, biosphere, and lithosphere. The carbon cycle takes many forms. Carbon helps regulate the amount of sunlight that enters Earth's atmosphere. It is exchanged through photosynthesis, decomposition, respiration, and human activity. Carbon that is absorbed by soil as an organism decomposes, for example, may be recycled as a plant releases carbon-based nutrients into the biosphere through

photosynthesis. Under the right conditions, the decomposing organism may become peat, coal, or petroleum before being extracted through natural or human activity.

Between periods of exchange, carbon is sequestered, or stored. The carbon in fossil fuels has been sequestered for millions of years. When fossil fuels are extracted and burned for energy, their sequestered carbon is released into the atmosphere. Fossil fuels do not re-absorb carbon.

In contrast to fossil fuels, biomass comes from recently living organisms. The carbon in biomass can continue to be exchanged in the carbon cycle.

In order to effectively allow Earth to continue the carbon cycle process, however, biomass materials such as plants and forests must be sustainably farmed. It takes decades for trees and plants such as switchgrass to re-absorb and sequester carbon. Uprooting or disturbing the soil can be extremely disruptive to the process. A steady and varied supply of trees, crops, and other plants is vital for maintaining a healthy environment.

Algal Fuel

Algae is a unique organism that has enormous potential as a source of biomass energy. Algae, whose most familiar form is seaweed, produces energy through photosynthesis at a much quicker rate than any other biofuel feedstock—up to 30 times faster than food crops!

Algae can be grown in ocean water, so it does not deplete freshwater resources. It also does not require soil, and therefore does not reduce arable land that could potentially grow food crops. Although algae releases carbon dioxide when it is burned, it can be farmed and replenished as a living organism. As it is replenished, it releases oxygen, and absorbs pollutants and carbon emissions. Algae takes up much less space than other biofuel crops. The U.S. Department of Energy estimates that it would only take approximately 38,850 square kilometers (15,000 square miles, an

area less than half the size of the U.S. state of Maine) to grow enough algae to replace all petroleum-fueled energy needs in the United States.

Algae contains oils that can be converted to a biofuel. At the Aquaflow Bionomic Corporation in New Zealand, for example, algae is processed with heat and pressure. This creates a "green crude," which has similar properties to crude oil, and can be used as a biofuel.

Algae's growth, photosynthesis, and energy production increases when carbon dioxide is bubbled through it. Algae is an excellent filter that absorbs carbon emissions. Bioenergy Ventures, a Scottish firm, has developed a system in which carbon emissions from a whiskey distillery are funneled to an algae pool. The algae flourishes with the additional carbon dioxide. When the algae die (after about a week) they are collected, and their lipids (oils) are converted into biofuel or fish food.

Algae has enormous potential as an alternative energy source. However, processing it into usable forms is expensive. Although it is estimated to yield 10 to 100 times more fuel than other biofuel crops, in 2010 it cost $5,000 a ton. The cost will likely come down, but it is currently out of reach for most developing economies.

People and Biomass

Advantages

Biomass is a clean, renewable energy source. Its initial energy comes from the sun, and plants or algae biomass can regrow in a relatively short amount of time. Trees, crops, and municipal solid waste are consistently available and can be managed sustainably. If trees and crops are sustainably farmed, they can offset carbon emissions when they absorb carbon dioxide through respiration. In some bioenergy processes, the amount of carbon that is re-absorbed even exceeds the carbon emissions that are released during fuel processing or usage. Many biomass feedstocks, such as switchgrass, can be harvested on marginal lands or pastures, where they do not compete with food crops. Unlike other renewable energy sources, such as wind or solar, biomass energy is stored

within the organism, and can be harvested when it is needed.

Disadvantages

If biomass feedstocks are not replenished as quickly as they are used, they can become non-renewable. A forest, for instance, can take hundreds of years to re-establish itself. This is still a much, much shorter time than a fossil fuel such as peat. It can take 900 years for just a meter (3 feet) of peat to replenish itself.

Most biomass requires arable land to develop. This means that land used for biofuel crops such as corn and soybeans are unavailable to grow food or provide natural habitats.

Forested areas that have matured for decades (so-called “old-growth forests”) are able to sequester more carbon than newly planted areas. Therefore, if forested areas are not sustainably cut, re-planted, and given time to grow and sequester carbon, the advantages of using the wood for fuel are not offset by the trees’ regrowth. Most biomass plants require fossil fuels to be economically efficient. An enormous plant under construction near Port Talbot, Wales, for instance, will require fossil fuels imported from North America, offsetting some of the sustainability of the enterprise. Biomass has a lower “energy density” than fossil fuels. As much as 50% of biomass is water, which is lost in the energy conversion process. Scientists and engineers estimate that it is not economically efficient to transport biomass more than 160 kilometers (100 miles) from where it is processed. However, converting biomass into pellets (as opposed to wood chips or larger briquettes) can increase the fuel’s energy density and make it more advantageous to ship.

CONCLUSION

Abundant amount of biomass is being wasted every year in agriculture country like Pakistan, which is already facing huge number of problems including fuel shortage. Majority of biomass can be converted into many of bio products including biofuels that will fulfill shortage of both LPG and CNG. Therefore, it is important to convince farmer that burning of biomass in their agriculture fields are releasing carbon monoxide, carbon dioxide, nitrogen oxides, and other pollutants. If these pollutants are not

captured and recycled, burning biomass can create smog and even exceed the number of pollutants released by fossil fuels. These are main factors those are damaging our environment and making climatic disasters in Pakistan.