Special Issue July 2021

ISSN Print: 2518-4261
ISSN Online: 2518-427X
Vol. 58(S), July 2021

PROCEEDINGS
OF THE PAKISTAN ACADEMY OF SCIENCES:
B. Life and Environmental Sciences

PAKISTAN ACADEMY OF SCIENCES
ISLAMABAD, PAKISTAN

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Other Countries: US$ 200.00 (includes air-lifted overseas delivery)

HEC Recognized, Category Y

Published by **Pakistan Academy of Sciences**, 3-Constitution Avenue, G-5/2, Islamabad, Pakistan
Tel: 92-51-9207140; **Fax:** 92-51-920 6770; **Websites:** www.paspk.org/proceedings/; www.ppaspk.org

Printed at **Graphics Point.,** Office 3-A, Wasal Plaza, Fazal-e-Haq Road, Blue Area, Islamabad, Pakistan
Ph: 051-2806257; E-mail: graphicspoint16@gmail.com

PROCEEDINGS
OF THE PAKISTAN ACADEMY OF SCIENCES:
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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 1-10 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)651

Pakistan Academy of Sciences

Review Article

Functional Food Plants and their Potential Antiviral and Immunomodulatory Properties: the Covid-19 Perspective

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Abstract: The pandemic of coronavirus disease (Covid-19) which is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is continuously hitting the world and millions of individuals have been affected so far. Limited therapeutic options are available for the treatment of Covid-19 while scientists around the globe are working hard to make the vaccines clinically available to the maximum human population. Alarmingly, SARS-CoV-2 variants are emerging in different regions of the world, hence threatening the efficacy of the clinically available vaccines. In such a scenario, the utilization of medicinal plants or traditional medicine could be the most preferred choice along with the precautionary measure to be adopted against the Covid-19. The current article has summarized few important food plants that have previously exhibited promising immunomodulatory or antiviral activities. These medicinal plants could be suggested for boosting the immune system and could be utilized against their utilization against SARS-CoV-2. It could be concluded that medicinal plants especially *Allium sativum, Curcuma longa,* and *Allium cepa* along with other plants/herbs/spices could not only be used against SARS-CoV-2 but also other viral, bacterial, or other parasitic diseases other prevalent diseases prevalent in the region.

Keywords: Medicinal plants, Antivirals, Immunity boosters, Covid-19.

1. INTRODUCTION

The second wave of coronavirus disease-19 (Covid-19) pandemic has been experienced around the globe continued to affect millions of individuals across the globe [1] the situation is further proceeding towards 3rd and 4th wave. The Covid-19 which is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was initially originated from Wuhan city of China in late December of 2019. Coronaviruses belonging to the *Coronaviridae* family are positive sense, enveloped single-stranded RNA viruses, and have a genome that ranges from 26 to 32 kb in length [2]. Coronaviruses have been reported both in avian hosts and numerous mammals, which include bats, masked palm civets, dogs, and camels, and were initially considered to be pathogens causing moderate to severe diseases in immunocompetent individuals until coronavirus emerged in 2002 inducing SARS-CoV outbreak [3-6]. There are seven species of coronaviruses currently known to infect humans and can cause common or/and serious diseases. For more than 30 years, HCoV-229E, OC43, NL63, and HKU1 have been the only existent human coronaviruses, causing only mild symptoms of common cold, respiratory tract illness, and pneumonia [7, 8]. The remaining three coronaviruses, named SARS-CoV (emerged in 2002-2003, led to the spread of SARS and cause serious illness) and the MERS-CoV appeared in Saudi Arabia in 2012, caused infection in humans and camels [9]; SARS-CoV-2 which emerged in 2019 in Wuhan, China, spread throughout the world (and serious efforts are being made to control its outspread) [10, 11].

Received: February 2021; Accepted: July 2021
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For the treatment of Covid-19, several therapeutic approaches have been suggested such as anti-inflammatory drugs, Lopinavir/Ritonavir, nucleoside analogs, etc. These drugs could be clinically effective against other infections however, their clinical usefulness in Covid-19 still needs to be explored [12, 13]. The food and drugs regulatory administration (FDA) approves a drug and its treatment mechanism after ensuring its safety from injection to ejaculation from the body. Consequently, instead of scratching a completely new drug and investing time and lives, plant-based antiviral treatments against the viruses like SARS-CoV-2 have been suggested. Considering the structure and infection strategies the virus adopts, various phenomenon can be used as targets for treatment including receptor binding and membrane fusion inhibition, viral RNA synthesis inhibition, virus-specific enzyme inhibition, exocytosis inhibition of new viruses from the host cell, and so on. With the help of therapeutic anti-inflammatory drugs, post-viral respiratory symptoms are tried to be diminished [13].

For centuries, in almost all cultures around the world, medicinal plants have been used for the treatment of several diseases including viral, bacterial, fungal, and other infections [14, 15]. Medicinal plants have been extensively reported to have high antioxidant, antiviral, antibacterial, antifungal, and other biological activities. We have summarized some medicinal plants and their bioactive compounds reported against respiratory viruses in Table 1. Bioactive compounds such as alkaloids, tannins, flavonoids, phenolic diterpenes, and other phenolic compounds have been isolated from plants [15-19]. Similarly, functional and nutraceutical foods have been studied and attempts have been made to scientifically validate their health improving potential [19, 20].

The current article was aimed to document medicinal plants and indigenous resources to fight viral infections, particularly accessible food plants that have been reported to be biologically active against respiratory tract infections. Developing countries like Pakistan are continuously affected by several diseases particularly viral infections such as dengue, HCV, polio, HIV, along with bacterial, fungal, and parasitic infections [21-28]. The less-developed infrastructure and limited health facilities could make the situation worsen especially in conditions like the current pandemic of Covid-19. Therefore, several important medicinal plants with promising antiviral and immunomodulatory properties need to be utilized. The current review could also help the public/researchers to increase the uses of medicinal food plants hence utilizing both the therapeutic effects as well as potential preventive effects, especially boosting the immune responses to infections.

2. COMMON ROUTINELY USED FUNCTIONAL FOOD PLANTS

For a long time, human are cultivating and using plants not only as a food source but also as therapeutic agents for several diseases. Plants having impressive immunomodulatory effects and therefore have grabbed the attention of researchers to explore such bioactive compounds in plants. Several compounds with incredible immunomodulatory properties have been reported including polysaccharides, flavonoids, terpenoids, and alkaloids. Importantly, these compounds possess comparatively fewer adverse effects than allopathic medicines [29]. The routinely used food plants with immunomodulatory effects and/or antiviral effects include the following plants.

2.1 *Allium cepa* L. (Onion)

Allium cepa L. which is commonly known as the onion is famous for its taste in flavors mostly used in salad. Onions contain compounds such as quercetin which could help reduce blood pressure, increase superoxide production, and hence increase the bioavailability of nitric oxide [30]. Potential antioxidant activities of onion have also been reported as onion contains flavonoids and sulfur compounds. Antiviral activities of compounds present in onions have also been reported [31, 32]. Flavonoids have been known to be very effective against viruses as they either inhibit or kill the viruses [33-36]. Flavonoids inhibit viral growth by inhibiting the synthesis of viral nucleic acids and proteins [37-39]. Phytochemicals such as kaempferol and quercetin found in onion play a vital role in inhibiting viral growth while also exhibit virucidal activities [40]. The virucidal activities have been reported against herpes simplex type I virus, rabies virus, polio virus, mengo virus, pseudorabies virus, sindbis virus, and parainfluenza type 3 virus [41, 42]. It has also been reported that quercetin could inhibit the replication of several respiratory viruses hence reducing viral load [31, 32]. We suggest that *Allium cepa* L. can be further investigated for its anti-SARS-CoV-2 properties.

2.2 *Allium sativum* L. (Garlic)

Garlic has been extensively studied for its tremendous biological activities and has been a popular herbal remedy for centuries. Studies have reported the beneficial effect of garlic on immune systems along with its antiviral properties as it is a source for Sulphur containing compounds and polyphenols [43-45]. It has been reported that proteins in garlic improve the activities of human peripheral blood lymphocytes and natural killer cells; resulting in increased immunity against viral infections. Mitogenic activities of garlic compounds on lymphocytes, basophils, and mast cells have also been observed [46, 47]. Garlic has tremendous immunostimulatory effects and could be used for boosting immunity and enhancing host resistance [15]. Because of such biological activities, garlic is recommended to be used in the current pandemic crises.

2.3 *Curcuma longa* L. (Turmeric)

Turmeric is a spice that has been used in traditional medicine and is an integrated part of Asian cooking and culture. In turmeric, curcumin is present which possesses antimicrobial, anti-inflammatory, wound-healing, antioxidant, hypo-glycaemic, chemo-preventive and several other properties [48, 49]. Turmeric has been widely used as a household remedy for treating sore throat, respiratory illness, and cough as in Asia. Antiviral properties of curcumin derivatives have been reported against infections of influenza viruses [50, 51]. The beneficial effects of curcumin against other viruses particularly against human immunodeficiency viruses have been reported. Antiviral activities of turmeric against chikungunya, dengue, hepatitis B virus, and hepatitis C virus had also been observed. With a good safety profile, turmeric has been used for centuries and its promising activities against the influenza virus could support its uses against other respiratory viruses particularly SARS-CoV-2, however, well-defined randomized studies for its way along with the value of usage are needed.

2.4 *Camellia sinensis* (L.) Kuntze (Tea plant)

Camellia sinensis (L) Kuntze is very important plant, and black/green tea is being made from its leaves for thousands of years. Interestingly, after water, tea is the second most consumed beverage in the world [52]. Its consumption has been associated with beneficial effects against inflammation, diabetes, vomiting, cardiac ailments, and cancer. Black tea has been investigated to have increased lympho-proliferative action when applied to cultured human peripheral mononuclear cells [53]. The immunomodulatory properties of green tea have been observed due to the presence of quercetin, gallic acid, and epigallocatechin [54]. Other compounds such as tannic acid, catechins, isotheaflavin-3-gallate have been observed to have 3CL protease activities which suggest the potential use of tea plants against the infection of SARS-CoV-2 and other respiratory viruses which are a major threat to the human population globally [55]. Locally, black tea (containing sugar particularly black/brown sugar) is routinely used against different types of respiratory illnesses. However, there is no evidence of this remedy to be effective against SARS-CoV-2.

2.5 *Glycine max* (L.) Merr. (Soybean)

Several important bioactive components of soybean have exhibited antiviral activities against several viruses [56, 57]. Isoflavones from soybean could be beneficial in immune response in viral challenging conditions. Isoflavones have been reported to reduce the infectivity of rotaviruses [57]. Flavonoids from soybean have also modulated the infectivity of viruses such as herpes simplex viruses while genistein has exhibited inhibitory activities against cytopathic effects which are structural changes in host cells caused by a viral infection and leads to cell death [58]. Such bioactive compounds from soybean possess several applications in the human health sector particularly the soy-derived isoflavones that have a potential immunomodulatory effect and could be helpful in the current Covid-19 pandemic.

2.6 *Mangifera indica* L. (Mango)

Mango is a fruit-producing as well as a medicinal plant and has been known for its therapeutic uses. The mango fruits have been documented as a vital source of vitamins, micronutrients, and other phytochemicals [59]. The immunomodulatory activities of mango have been widely reported [60]. The methanolic extract of mango has been associated with an increase in humoral antibody titers and enhanced delayed-type hypersensitivity which confirms its immunomodulatory activities [61]. Further, the hexane leaves extract of mango has been reported to increase the white blood cell count along with spleen and thymus size which further confirm its immunomodulating properties via white blood cells [62]. The antiviral activities of mangiferin have also been reported and were found to act as a potent biological modifier [63]. Antiviral activities of mangiferin against herpes simplex viruses, human immunodeficiency virus, hepatitis B virus have been reported [63-65]. The immune-modulatory properties of mango could also help alleviate Covid-19.

2.7 *Abelmoschus esculentus* L. (Okra)

Okra has been distributed in various parts of the world. The fruits of okra have been reported with promising immunomodulatory activities [66-69] The okra flowers are also expected to be a potential source of polysaccharides with immune-stimulatory properties. Besides the consumption of okra pods as foods, it has also been used as traditional medicine for the treatment of several diseases. The okra pods have been used to treat diarrhea and dysentery in acute stomach inflammation, kidney catarrhal infections, dysuria, ardor urine, and bowels. The infusion of okra roots has also been used in syphilis treatment while the juice of the roots was used for treating wounds, boils, and cuts [70]. Researchers have recently reported that polysaccharides from okra exhibited significant macrophage stimulatory activities [71]. They also reported the immunomodulatory activities, increased spleen and thymus index, and promoted cytokines production [71]. However, *Abelmoschus esculentus* needs to be further explored for its potential anti-SARS-CoV-2 properties.

3. SPICES AND HERBS AS PROMISING ANTIVIRALS

Although the previous section has discussed a few herbs/spices along with their potential biological activities particularly antiviral and immunomodulatory activities. However, there are several other herbs and spices which exhibited promising antiviral activities. Several herbs/plants such as *Ocimum basilicum* (Tulsi), *Allium sativum* (garlic), and *Tinospora cordifolia* (Giloy) along with many others are well known for their tremendous immunity booster properties [72]. Spices such as ginger, turmeric, cinnamon, clove, and black pepper are famous for their antiviral and immunity booster properties. These herbs could be very helpful for the treatment of Covid-19.

The commonly available medicinal plant includes ginger which belongs to the Zingiberaceae family. Other famous members of this family are cardamom, galangal, and turmeric. Several bioactive compounds such as steroids, alkaloids, and phenolic compounds are well known for their medicinal properties are present in ginger. Several other sub-compounds which are well known for their antipyretic, anti-arthritic, anti-inflammatory and antiemetic activities have also been reported from ginger [73]. More importantly, the bioactive compounds from ginger have exhibited tremendous antiviral activities against several viruses particularly respiratory viruses including SARS and influenza viruses [74, 75].

Cinnamomum cassia is commonly known as cinnamon another important medicinal plant widely used as traditional Chinese, Persian, Unani, and Indian medicines. Cinnamon has been reported as a source of compounds with tremendous antiviral, antioxidant, antidiabetic, antitumor, antihypertensive, and antimicrobial activities [76].

Black pepper which is famous for its pungent smell has significant biological properties. Several compounds from black pepper are being used in the medicine and perfume industry. An important compound i.e alkaloid piperine from black pepper is known for promising pharmacological properties such as anti-inflammatory, antipyretic, antitumor, and antimicrobial activities [77, 78]. Antiviral activity of chloroform and methanolic extract of black pepper against human parainfluenza and vesicular stomatitis virus has also been reported [79]. Bioactive compounds such as piperdardiine and piperanine are suggested for the treatment of

Table 1. Reported medicinal plants having potential efficacy against respiratory viruses.

S. No.	Medicinal Plant	Family name	Reported against viruses	References
1.	*Aesculus chinensis*	Sapindaceae	Influenza	[86, 87]
2.	*Allium cepa* L	Amaryllidaceae	Influenza	[41]
3.	*Argimonia Pilosa*	Rosaceae	Influenza	[80]
4.	*Artemisia annua* L.	Asteraceae	SARS-CoV	[102]
5.	*Blumea laciniate*	Asteraceae	Respiratory syncytial virus	[89]
6.	*Brazilian propolis*	Asteraceae	Influenza	[98, 99]
7.	*Camellia sinensis*	Theaceae	HCV, influenza	[81]
8.	*Curcuma longa*	Zingiberaceae	Influenza	[97]
9.	*Eleutherococcs senticosus*	Araliaceae	Influenza virus A	[90]
10.	*Geranium sanguineum*	Geraniaceae	Respiratory syncytial virus, Influenza	[88]
11.	*Glycyrrhiza glabra*	Fabaceae	Influenza, SARS-COV	[85]
12.	*Lycoris radiata*	Amaryllidaceae	SARS-CoV, Influenza	[84]
13.	*Momordia charantia*	Cucurbitaceae	Influenza	[91]
14.	*Nerium indicum*	Apocynaceae	Influenza, HSV	[92, 93]
15.	*Piper nigrum*	Piperaceae	Influenza	[79]
16.	*Radix glycyrrhiza*	Fabaceae	SARS-CoV-2	[82, 83]
17.	*Scutellaria baicalensis*	Lamiaceae	Influenza	[94-96]
18.	*Urtica dioica*	Urticaceae	Influenza	[100, 101]
19.	*Verbescum thapsiforme*	Scrophulariaceae	influenza viruses	[103]

Covid-19 [19]. Several of the above-mentioned plants are being used as household remedies against viral infections especially those affecting the respiratory and/or digestive tract. The global problems could be addressed via local solutions and the indigenous resources need to be utilized in the current Covid-19 pandemic.

4. CONCLUSION

Medicinal plants are a promising alternative for the prevention and treatment of various diseases. China has utilized several medicinal plants as traditional medicines since the start of Covid-19. Several spices, herbs, and other medicinal plants are cultivated in Pakistan on large scale. Developing countries like Pakistan are continuously experiencing disease outbreaks (both viral and bacterial) and the current pandemic of Covid-19 could further aggravate the situation due to a large number of cases and co-infections [22]. In the current Covid-19 pandemic, the use of immune boosters and precautions could be the best options. The use of medicinal plants particularly spices and other food plants possesses important antiviral activities. Because of the presence of potential antimicrobial, immunostimulatory and immunomodulatory, antivirals, and other important properties, the mentioned medicinal plants are not only recommended in the current situation but also need to be used against other infections prevalent in the country. These medicinal plants might have side effects if used inappropriately, therefore, further studies are needed to explore their effectiveness, amount of consumption along with the mode of consumption, and/or way of administration.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 11-17 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)652

Pakistan Academy of Sciences

Review Article

COVID-19 Vaccines: Pakistan's Perspective

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Abstract: The pandemic of COVID-19 has affected millions of individuals around the globe. Its impact on the world has made essential progress in the research sector to develop safe and effective vaccines. Several vaccines for COVID-19 have been made utilizing the SARS-CoV-2's spike protein. Presently, Moderna's COVID-19 vaccine, Pfizer-BioNTech COVID-19 vaccine, and Johnson & Johnson's Janssen vaccine are approved and suggested by CDC to prevent the COVID-19. Five vaccines (till April 2021) have been approved by WHO on emergency basis which are AstraZeneca, Pfizer-BioNTech, Moderna, Sinopharm, and Johnson & Johnson. The Food and Drug Administration approve the scientific standard of drugs and vaccine such as their efficacy, safety, and quality. Currently, ambiguous information regarding COVID-19 vaccine are being circulated globally. During health crisis, rumours roll out and generate fear, psychosis, and anxiety. On the other hand, the variants of SARS-CoV-2 are continuously emerging across the globe. Different platforms are being utilized for the development of whole virus-based vaccine, nucleic acid-based vaccine, and proteins sub-unit vaccine; all displayed good efficacy where few were further proceeded to clinical trials. The current article provide an overview on the COVID-19 vaccines, their efficacy, and discuss the possible reduction in vaccine efficacy due to the emergence of new variants.

Keywords: COVID-19, Vaccine, New Variants, Pandemic, Efficacy, Mutations.

1. INTRODUCTION

The COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has affected millions of individuals around the globe [1, 2]. Scientists across the globe are trying to develop vaccines that are both effective and safe to prevent COVID-19. Several candidate vaccines are in the pipeline or already in the clinical trial developmental stages while some or clinically available and have been approved. Studies from animal models along with human trials already indicated potential trends to achieve a high level of neutralizing antibodies [3]. Promising results have been revealed by the antibodies of SARS-CoV-2 spike proteins in terms of inducing high titers in preclinical models.

A proteins-based recombinant vaccine such as CoV-RBD219N1 and chemically inactivated virus vaccines i.e PiCoVacc have reported a high level of protective immunity in animal models such as mice and rhesus macaques [4, 5]. Other studies have reported adenovirus vectored vaccines as inducing high-level of antibodies for SARS-CoV-2 [6,7]. The mentioned vaccine of COVID-19 (ChAdOx1-nCoV-19) has shown antibodies titers ranging from 5-40 in the rhesus macaques [6]. A human adenovirus 5-vactored COVID-19 vaccines in trial phase-I induced both live virus-neutralizing antibodies titers and pseudovirus neutralizing antibodies titers in healthy individuals in 28 days post-vaccination [7]. The reported titers of antibodies induced by adenovirus vectored COVID-19 vaccines were lower than the reported human convalescent plasma [8, 9].

Efforts have been made to develop vaccines candidates particularly utilizing the SARS-CoV-2's spike protein [10]. Public health authorities across

Received: February 2021; Accepted: July 2021
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the globe have been mobilizing to deliver the biggest ever vaccination program to battle COVID-19. As of 25th May 2021, 5.3% of the world population has been fully vaccinated [11]. Presently, Moderna's COVID-19 vaccine, Pfizer-BioNTech, and Johnson & Johnson's Janssen COVID-19 vaccine are approved and suggested by CDC to prevent COVID-19 [12, 13]. As of 27th February 2021, there are almost 50 other COVID-19 vaccines still in developmental stages [11, 12]. Five vaccines have been approved by WHO on emergency basis which are AstraZeneca, Pfizer-BioNTech, Moderna, Sinopharm, and Johnson & Johnson's Janssen [14].

Vaccines could be based on live or attenuated viruses, virus-like particles, proteins sub-unit, viral vector, DNA, RNA, and/or conjugated nanoparticles [15]. The mRNA-based vaccines trigger an immune response as they teach the cells how to make a protein that could activate the immune response. The Pfizer-BioNTech which is an mRNA-based vaccine is recommended by CDC for people aged 16 years and older. Similarly, the Moderna vaccine is also a mRNA-based vaccine and is recommended for people aged 18 years and older. The BNT162b1vaccine is an mRNA vaccine that encodes for the SARS-CoV-2 RNA binding domain (optimized codon) which is an essential target for neutralizing antibodies. The vaccine immunogenicity has been enhanced by the addition of T4 fibritin-derived fold on trimerization domain to the RNA binding domain. The mRNA is efficiently delivered as it is encapsulated in 80nm ionizable cationic lipid nanoparticles. The clinical trials have revealed moderate to transient reactions with no obvious adverse side effects [16].

The mRNA-1273 is a synthetic mRNA vaccine encapsulated in the lipid nanoparticles encoding for spike proteins of SARS-CoV-2. This vaccine could activate the immune response to the spike protein and is considered safe because of its mRNA nature [17]. This vaccine was quickly approved by the food and drug administration (FDA) for clinical trials [18]. The vaccine was reported to be welltolerated and safe in 25-100 µg dose cohorts [19].

1.1 Vaccine Authorization and Policies

The FDA approve the scientific standard of drugs and vaccine such as their efficacy, safety, and quality. FDA also provides regulatory and scientific advice to researcher and vaccine developers and evaluate the information of clinical phases of vaccines [20]. FDA enhanced the process of the vaccine's approval due to the public health emergency and the importance of the urgent need and availability. The interim final rule with request for comments (IFC) discusses CMS implementation of section 3713 of the Coronavirus relief, Aid, and Economic-Security Act (CARES Act) which is recognized as Medicare Part-B coverage and payment for COVID-19 vaccine and its administration [20].

1.2 Vaccines in Pakistan

Pakistani health authroities have awarded emergency use authorization to COVID-19 vaccines particularly the Russian vaccine, Sputnik-V, or Gam COVID Vac to be administered in Pakistan. The authorities have also granted authorization to China's state-owned Sinopharm and British-Swedish-vaccine manufacturers Oxford AstraZeneca's AZD1222/ Covisheild vaccine [21, 22]. COVID-19 vaccination has been started in

Table 1. Vaccines that clinically approved or have entered to clinical trial (Phase III) [12]

S. No.	Vaccines	Producer Company/ Organization
1	Ad5-nCoV	CanSino Biologicals
2	INO-4800	Inovio, Inc
3	mRNA-1273	Moderna
4	ChAdOx1	University of Oxford
5	Pathogen-specific aAPC	ShinzenGeno-Immune Medical Institute
6	BNT162b2	Pfizer, Inc., and BioNTech

Pakistan. The emergency use authorization was announced by the Drug Regulatory Authority on 22nd January of 2021 following which vaccines could be shifted to the country in the coming few days [22]. The 5 million doses of vaccine have been donated by China and 17 million vaccines have been donated by the UK to Pakistan.

2. MISINFORMATION ASSOCIATED WITH VACCINE IN PAKISTAN

Ambiguous information regarding COVID-19 are being circulated across the globe. Keeping vaccines at the center of conflict, many myths and rumors have been spread. Vaccines associated with infertility were the most heard rumor following by vaccines ingredients and their composition used for vaccine harming the person who inject it. A huge part of the world suffering from this pandemic has faced the hardest challenges to coping with the loss of loved ones [23]. The reason for the uncertainty associated with the use of vaccination is also due to certain factual cases related to COVID-19 vaccination e.g., the certain patient developed thrombotic thrombocytopenia (blood clotting) after receiving the AstraZeneca vaccine. More than 20 million people have been vaccinated (as per April 2021) with the AstraZeneca vaccine in the UK and about 79 cases of rare blood clots with low platelets have been reported along with 19 deaths. However, the WHO and the European Medicines Agency recommended that there is no increased risk of blood clots with the vaccine and vaccinations should be continued [24]. People also urged that the vaccine-based immunity is not long-lasting, hence there could be chances of reinfections and vaccination is not an appropriate option.

During a health crisis, rumors roll out and generate fear, psychosis, and anxiety. Patients with HIV, cancer, organ transplants, bone marrow recipients with the suppressed immune system are not willing to get vaccines [25]. Other misconception also exists according to which tracking device/chip is implanted in the human body [26]. The spread of coronavirus rumors gives rise to serious menace not only for the effectiveness of vaccine campaigns but also to public health. Vaccines are only a part of efforts to put pandemics under control, adding that it is also important for the authority to increase testing, tracing, and treatment [27].

2.1 New Variants Reported from Pakistan

Fearfully, VOC-202012/01 (Variant of Concern, the year 2020, month 12, variant 01) formerly known as VUI-202012/01, was recognized in United Kingdom, South Africa, and Brazil [28]. About 31 countries of the world have reported the new variants till 30 December 2020 [29]. It is thought that new virus might be up to 70% more transmissible and threaten than old virus strain, which could cause another wave of the same pandemic. According to a newly published study, a new coronavirus variant moved from the UK to the US rapidly [30]. In Pakistan's first case of the UK variant was reported in Sindh province. On 29th December 2020 in Karachi, three samples of UK returnees show 95% similarity in the first phase of genotyping to new coronavirus variants from the UK. It has been documented that three patients of UK returnees have a new variant of SARS-CoV-2 in the first phase, 12 samples were genotyped in which six were detected positive. Besides, the National Command and Operation Centre (NCOC) reported two positive cases of the UK variant in Islamabad on 4th, January 2021. The individuals had recently returned from the UK while the variant was confirmed via whole-genome sequencing. Pakistan has extended the travel restrictions on several countries, including the UK, till February 28 to minimize the spread of the deadly variant of the coronavirus amidst the second wave of infections [31].

3. EFFICACY OF VACCINE COVID-19

It has been documented that the vaccine formed by the Chinese (Cansino Biologic's COVID-19 vaccine) was 65.7% effective in averting the symptomatic cases and 90.1% in severe disease [29]. The percentage was based on analysis of multiple countries' phase-III clinical trials of the Cansino vaccine, however, the Pakistani sub-set with 30,000 contestants exhibited 74.8% defense against symptomatic cases and 100% in serious illnesses. In addition to Pakistan, Cansino has analyzed its vaccine in Chile, Mexico, Russia, and Argentina. Neither the Cansino nor Pakistani government announced further information on the efficacy statistics, suggesting that Cansino joins other Chinese vaccine manufacturers Sinopharm and Sinovac in publishing little data beyond headline efficacy figures [30]. The early trials showed that Cansino's vaccine induced only a limited immune response that was surpassed by Pfizer and Moderna. After rising in the vaccine run, foreign vaccine manufacturers like Moderna and Pfizer also defeated Cansino in completing Phase-III clinical trials, obtaining approvals from the government. Cansino also fell behind its Chinese counterparts Sinovac and Sinopharm, which are now distributing hundreds of millions of doses across at least a dozen foreign countries. Sinopharm's vaccine is 79% effective while Sinovac's vaccine is between 50% and 90% effective [32].

Cansino's shot is based on similar viral-vector vaccine technology that Johnson & Johnson is using for its one-shot vaccine. Cansino's 65% efficacy rate also appears on par with Johnson & Johnson's 66% figure. Cansino's 65% efficacy figure would pass the WHO's recommended threshold of 50%, but it still lags the 94.1% and 95% figures posted by Moderna and Pfizer, respectively [33]. Like the Johnson & Johnson single-dose vaccine, Cansino may have distinct advantages in distributing its jabs to poor and middle-income countries. Unlike mRNA vaccines from Pfizer and Moderna, Cansino's vaccine does not require sub-zero storage; it can be transported in less expensive supply-chain networks at normal refrigerated temperatures (2 to 8 degrees Celsius) [32,34]. Its administration in a single dose could boost efforts to distribute vaccines to more rural areas, where it may be difficult to send supplies and set up follow-up appointments. Cansino's vaccine has not been officially approved in Pakistan or any other country, but it has been distributed to members of China's military and other high-risk population groups in China on an emergency basis since at least June 2020. In November 2020, Cansino has the potential benefits of having a viable one-shot COVID-19 vaccine. In the pandemic environment, what you need is a vaccine that can quickly provide protection. If we can make a single dose work, it will stop the spread of the virus [32, 35].

3.1 Possible Reduction in Vaccine Efficacy due to Variation

The Oxford-AstraZeneca vaccine shows good efficacy against the UK's dominant variant (new corona-virus variant). Vaccine designers declare it is a comparatively simple process to adjust the existing formula to target any new variants. Scientists behind the Moderna and Pfizer-BioNTech vaccines also suggest their vaccines seem to protect against UK's dominant new variant. Now that more than 10 million people have been vaccinated, these are the first indications that the vaccine even now defends maximum people with COVID-19. Oxford investigators reveal similar levels of efficacy against "Kent" B117 (74.6%) and the old variant (84%). It is now present across the UK and in other countries. In what way variants impact the severity of COVID-19 disease and how variants impact the efficacy of vaccines and therapeutics. Surveillance of emergent variants can assist detection of the variant with the ability to circulate more rapidly in people, capability to evade detection by specific diagnostic tests, ability to produce either moderate or more serious disease in people, ability to evade natural or vaccine-induced immunity, and reduced susceptibility to therapeutics that utilize monoclonal antibodies [36]. It has been stated that vaccines could work efficiently to avert COVID-19 and could not be affected by variations or the emergence of new SARS-CoV-2 variants. The report also reveals the vaccine may decrease the spread of the disease as well as preventing severe infection and death from COVID-19. The necessity for a new vaccine had always been estimated. "Corona-viruses are less disposed to mutation than influenza viruses, but due to continuing pandemic situation, we have always expected that new variants will begin to dominate and that ultimately need a new version of the vaccine for the updated spike protein. It would be essential to sustain vaccine efficacy at the highest possible level. There is a need for continuous monitoring on the emergence of new variants and work with AstraZeneca to make changes to the vaccine if compulsory [37].

The lineage carries many mutations in the SARS-CoV-2 spike protein, the immune system's prime target, which allows the virus to identify and infect host cells including some changes linked to weakened antibody activity against the virus. The rapid spread of variants could be its ability to elude previously established immune responses [38, 39]. To investigate this, a virologist isolated the new variant from infected people. Then they tested the variant samples against serum the antibody-containing portion of blood taken from six people who had recovered from COVID-19 caused by other versions of the virus. This convalescent serum tends to contain neutralizing, or virusblocking, antibodies that can prevent infection. The researchers found that the convalescent serum was much worse at neutralizing new variants than at neutralizing variants that circulated earlier in the pandemic [38]. Some people's plasma performed better against new variants than others, but in all cases, the neutralizing power was substantially weakened. The researcher probed the effects of convalescent serum on several groupings of spike mutations observed in variants. They did this using a 'pseudo-virus' a mutated form of HIV that infects cells using the spike protein of SARS-CoV-2. These experimentations demonstrated that variant contains mutations that blunt the effects of neutralizing antibodies that recognize two key regions of spike: its N-terminal domains and receptor-binding. Pseudo-viruses with the full package of new variants mutations were fully resistant to convalescent serum from 21 out of 44 participants and were partly resistant to the serum of many people [38, 40].

Mutations in the receptor-binding domain of variant caused a modest drop in the potency of antibodies from people who had received either the Pfizer or Moderna mRNA vaccines. Most COVID-19 vaccines elicit high levels of antibodies that target diverse regions of the spike protein, so some of the molecules are likely to be able to block variants of the virus. And other components of the immune response, such as T cells, might not be affected by new variants [38].

4. CONCLUSION

The development of a vaccine against COVID-19 was an important aspect and a challenging job. Presently, Moderna's COVID-19 vaccine and Pfizer-BioNTech COVID-19 vaccine are approved and suggested by CDC to prevent COVID-19. The vaccine formed by the Chinese resarchers was 65.7% effective in averting the symptomatic cases and 90.1% in severe disease. The emergence of new variants are due to the variation that occurs in the spike protein of SARS-CoV-2 and mutation in the receptor-binding domain which may causes a reduction in vaccine efficacy.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 19-30 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)656

Pakistan Academy of Sciences

Review Article

Plant Biotechnology; an Important Avenue for Medicine during Pandemics

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Abstract: The coronavirus pandemic that is still ongoing has let the world learn many lessons. One of the lessons is to search for a viable source of medicine against such viruses. Vaccines for those ailing with symptoms of such viruses are most importantly needed from a viable source. Plant biotechnology offers a platform called Biopharming, wherein vaccines are produced in a safe, biocompatible manner. These vaccines also promise to have the advantage of being produced cost-effectively once the complete map of the process is laid down and is upscaled. Vaccines are undoubtedly developed at an unprecedented rate and are the most effective strategy to fight such pandemics. However, as understood from a fundamental standpoint, the vaccine is a part of a preemptive strategy. Along with synthetic chemical compounds, phytochemicals cannot be overlooked as candidates for drugs against Severe respiratory coronavirus 2 (SARS-CoV-2). Compounds, for instance, *Glycyrrhizin* from the roots of *Glycyrrhiza glabra* have been shown as a very promising phytochemical against the SARS-CoV, which caused an outbreak in 2002-2003. Other chemical compounds, reserpine, emodin, betulonic acid, and apigenin isolated from different plants, were also effective against SARS-CoV. The production of these and many other compounds through plant biotechnology techniques such as transgenesis and gene editing followed by in vitro cultures is a vital avenue to be considered. Transgenesis offers the advantage of boosted production of existing phytochemicals or triggered production of novel compounds through in vitro cultures which serve as a reactor for the development of important phytomedicine. This article emphasizes the role of Biopharming in the production of vaccines against SARS-CoV-2 and any such future outbreak causing Virus or bacteria. The report also highlights the role of transgenesis and gene editing to produce medicine against the Virus.

Keywords: SARS-CoV-2, Pharming; plant biotechnology, in vitro cultures, transgenesis, Secondary metabolites

1. INTRODUCTION

The ongoing pandemic caused by the severe acute respiratory syndrome virus (SARS-CoV-2) has given a wake-up call to the world. Killing 2.6 million people and infecting 117 million across the globe so far, coronavirus disease (COVID-19) has become the deadliest disease in the past century [1]. When the SARS-CoV-2 was first identified in late 2019, scientists immediately geared up to start characterizing the Virus and looking for possible remedies against it. Owing to past scientific information and current robust scientific methods, the world was presented with five different vaccines against the coronavirus [2]. However, even though many countries are vaccinating their population against the Virus, the world is still lagging in tackling the pandemic [3].

Similarly, most currently approved vaccines are based on messenger RNA or nucleic acid isolated from the Virus [4]. These vaccines require a continued supply of many costly chemicals and reagents, along with the need for high maintenance requirements [5]. This calls for viable alternatives to vaccines. Plants offer one such platform for the production of medicine against the current and any potential pandemic virus [6]. Plants could produce secondary metabolites and other biomolecules with potent activity against viruses

Received: March 2021; Accepted: July 2021
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and bacteria [7]. However, plants too are subject to multiple constraints such as seasonal and geographical variations, non-uniform production of phytochemicals, and regulatory concerns.

Similarly, plants produce phytomedicine that is supposed to be used against symptoms caused by the Virus. While Vaccines, a preemptive strategy, are also needed in the fight against viruses. Plants offer pharming of vaccines as a strategy for producing safer vaccines [8]. Therefore, plant biotechnology is a very promising avenue to cope with the current and any future outbreaks. Tools of plant biotechnology that include transgenesis, gene editing, and pharming are the emphasis of this article which discusses their potential in detail. We aim to highlight the role of transgenesis and gene editing to produce medicine against the Virus.

2. PHARMING; AN AVENUE FOR VACCINES AGAINST COVID-19 AND OTHER PANDEMICS

Pharming or Biopharming entails the phenomenon of utilizing a living system to manufacture biological materials or drugs. Pharming employs living systems as bio-factories for the rapid and economically viable production of specific complex biomaterials on a high scale which in other cases may not be easily synthesized with already available manufacturing technologies. The first application of this approach was insulin production in 1978 by Genentech using a bacterial host Escherichia coli, which was subsequently commercialized in 1982 [8]. A technological leap was observed in Biopharming with the introduction of eukaryotic cells as production hosts for complex molecules, particularly those eukaryotic cells that have mammalian type post-translational modifications. Genentech, in 1987 commercialized the production of anticoagulant activase enzymes by remodeling *E.coli* fermenters for the production of Chinese Hamster Ovary (CHO) cells [8]. Following this development in technology, CHO cells were soon preferably utilized as hosts for extensive production of other complex biologics. In 2017, The estimated market worth of monoclonal antibodies was 123 billion USD, of which 87% mAb products were produced in CHO cells [9]. Biological materials production is currently dominated by fermentation-dependent technologies that generally take approximately 12 months to select clones, validating the required conditions and reaching the production capacity [10]. Non-Fermentation-based production of biological materials on an industrial scale is attributed to Transgenic animals, embryonated hen's eggs (EHE), and whole plants. Among these, whole plants need little input cost for biomass production and show the highest production capacity when a transient expression system is used [8]. Some of the strategies that could be used to employ pharming for the production of medicine against SARS-CoV-2 and any future outbreaks are shown in figure 1.

3. WHY PLANT BIOPHARMING FOR BIOLOGICS PRODUCTION?

Plant molecular pharming is a relatively novel idea for the production of proteins in plants. Molecular farming is a sub-discipline of plant biotechnology that uses certain plants as hosts to produce vital recombinant proteins, including vaccines, enzymes, and hormones [11]. Using plants for the production of diagnostic reagents and pharmaceutical proteins has been taking place for more than 30 years [12]. Molecular farming aims at the recovery and use of a particular recombinant protein rather than the plant itself. After playing its role as a host the plant is disposed of or used separately as a side stream, whereas the target protein is extracted, purified, and used for the purpose it was produced. Molecular pharming, in its inception, promised three advantages compared to other biologics, Low input cost required for growing plants, scalable production capacity, and insurance of product safety. This aroused the interest of researchers in the field and consequently resulted in an abundance of research publications for the expression of various proteins in plant-based expression systems. This development led to the establishment of several companies intending to commercialize this novel plant-based technology [13]. Plant molecular farming mainly encompasses using a whole plant such as cereals and tobacco, but the technology also invariably harness plant cells and tissue culture, aquatic plants, algae, moss, and performing in vitro plant-derived transcription and translation systems [8].

Low input cost and lesser biomass production requirements for plants compared to fermentation-

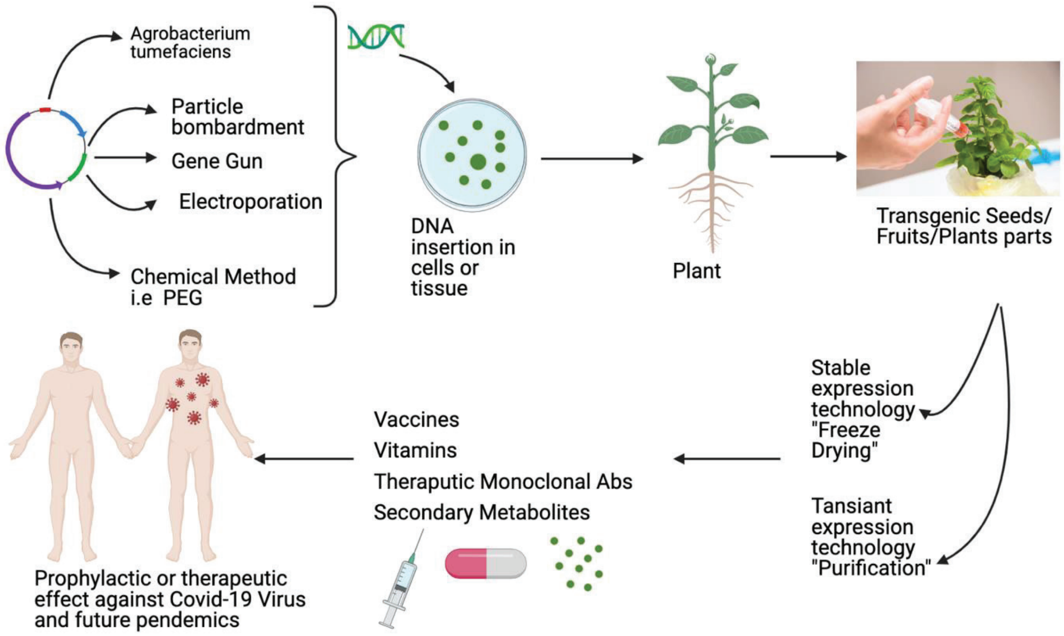

Fig. 1. Intervention points where transgenesis in plants will be of help to fight pandemics

based systems attracted the massive interest of researchers towards the field. Hence attempts were made to explore new prospects of using plants as low-price biofactories and using a carefully selected crop species will result in the production of edible vaccines. Efforts were made to scale plant-based biologics in crop plants such as rice, barley, maize, and safflower as production hosts [8]. Further investigations demonstrated production in plants based production systems in various other plants such as Sundews, moss, pitcher plants [14], corn, rice, barley, wheat, sunflower, tomato, soybean, carrot, lettuce, tobacco, tomato, Nicotiana benthamiana, and melon [15].

Molecular farming circumscribes several expression technologies that range from whole transgenic plant or more often a transient expression without the need for transgene integration [13]. In transient expression, an adult wild-type plant mostly tobacco—*Nicotiana benthamiana* or its relative *Nicotiana tabacum* is infiltrated with certain *Agrobacterium tumefaciens* strain or other viral vectors of the plant carrying that particular transgene [16]. The first generation of commercial biological products in the plant was centered on whole transgenic plants [17]. Currently, the arena of plant-molecular farming utilizes both transgenesis and transient expression strategies for entire plant production systems. Moreover, Systems based on cell cultures and plant-based systems are also in use [18]. The first genetically engineered plant-derived therapeutic approved by the FDA was manufactured by Protalix in 2012. Protalix biotherapeutics in Israel employed a transgenic carrot cell suspension system for the production of taliglucerase alfa, a drug for the treatment of an inherited metabolic disorder, Gaucher disease [19]. Although a broad range of plant-based systems has been tested under experiments, among them Nicotiana benthamiana is currently the host of choice for the production of biologics. The plant is a crucial production host of many Plant-based production companies such as PlantForm, Icon Genetics, Medicago, Capebio, iBio, Bioapp, and Leaf expression systems[20].

3.1 Plant Molecular Pharming during Times of Pandemics

In the past two decades, Viral outbreaks have strengthened the viewpoint that restraining an outbreak is best achieved with a faster detection system and spreading awareness about non-pharmaceutical interventions followed by immunization [21]. However, apart from the issues observed during the 2009 Pandemic of influenza A(H1N1), one of the main shortcomings observed was a global inadequacy to produce vaccines and inability to alleviate the spread due to the slow speed of production during the first wave. This was the result of dependence on an egg-based slow-yield vaccine manufacturing system [22].

Producing biological products in plant-based systems may serve as a practical arena for large-scale production within a couple of weeks, in contrast to longer periods required for production using cell-culture-based strategies [23]. A variety of plant species have been harnessed for antibodies, drugs, immunomodulatory proteins, vaccines, and biopharmaceuticals production, and they are regarded as living factories or bioreactors that inherently can produce biologics in a relatively short interval of time [24]. Vaccine against Newcastle disease virus (NDV) was first among plant-based vaccines to be approved for poultry by the United States Department of Agriculture (USDA), which demonstrated above 90% protection in poultry [25]. The only other product manufactured via a plant-based production approach to get licensed is the monoclonal antibody (scFv mAb). The antibody was harnessed to produce a recombinant Hepatitis B virus (HBV) vaccine in Cuba [26]. The fast tempo to respond to any viral outbreak was demonstrated by Mapp biopharmaceutical in the 2014 Ebola outbreak. The company established a quick production of an antibody cocktail against Ebola called ZMapp, which authorized an emergency approval for human use [27].

Molecular farming has resulted in several therapeutics and vaccines, to name a few vaccines production against the perilous Hepatitis B virus, cholera, and Dengue fever virus. The production of neutralizing monoclonal antibodies against HIV, Ebola virus, and therapeutic agent to provide treatment for those infected with Gaucher Disease [28].

VLP-based vaccines that have got approval are immunization against Norwalk virus, Bluetongue virus, Hepatitis B virus, and Papillomaviruses and up to 100 VLP-based vaccines are in the pipeline of clinical trials [29]. Lately, Medicago Inc has undergone phase III clinical trials of a quadrivalent nature plant-made-VLP vaccine. This development is considered a significant landmark in the production of plant-made biologics, and if it is approved for human use could promise explicit protection and a vast range of production options [30].

3.2 Services of Plant Molecular Pharming amid COVID-19 Pandemic

The outbreak of SARS-CoV-2 in December 2019 and its spread worldwide have created a myriad of challenges across the globe. These resultant challenges require efficient solutions in terms of public health and biomedical research [31]. This pandemic called for attention to specifically prepare and invest in those platforms that are more appropriate for flexible, quick, and environment-friendly production of medical remedies in terms of Diagnostics, Therapeutics, and vaccines against the emergence, re-emergence, and biological terrorism-related lethal diseases [29]. Plants have been consistently used for recombinant vaccine production for more than three decades and this phenomenon is termed “Molecular farming.” Therapeutics against COVID-19 in plants can be manufactured either by antibody expression against the Virus as passive protection or by the expression of SARS-Cov-2 antigenic components in plant-based expression systems [32, 33]. Vaccines produced in plant-based systems are generally termed third-generation vaccines. The procedure to manufacture a plant-made vaccine entails incorporating the candidate vaccine into a plant-based expression system. That plant expression promotes the candidate gene expression inside the plant machinery, which resultantly produces antigenic or protective protein.

The plant-based expression system serves as a bioreactor, producing the protein for many generations, thus ensuring an ongoing production and availability [23]. In addition, the SARS-CoV-2 Structural proteins, namely [Envelope (E), membrane (M), Nucleocapsid (N), and spike (S)] proteins elicit neutralizing antibodies(Nab) and activate cell-mediated immune responses [34].

Among structural proteins, the Virus uses the S protein to enter inside the cell via angiotensin-converting enzyme 2(ACE2) receptor binding. It thus makes S protein a tempting target to develop a vaccine against the Virus. The region of the S protein that interacts with the ACE2 receptor is RBD [23]. Bioinformatics-based epitope prediction through antigenic mapping of S protein has recognized essential immunogenic proteins that after expression in plants produces a vaccine against

SARS-Cov-2 [35, 36]. Two companies so far have proclaimed the development of antibodies and plant-based vaccines against COVID-19 causing viruses. A Canada-based company Medicago inc. had announced the production of Virus-like particles (VLPs) via a transient expression system soon after reaching out to the SARS-Cov-2 Spike (S) protein sequence [11]. The instant, continuous requirement for biologics during the COVID-19 and an observed inability of the current infrastructure to meet the demand has given rise to the perception of how a plant-based production system can help meet the immediate need for biologics [8]. Several plant-based biological drug manufacturing companies have started producing products related to SARS-Cov-2, using transient expression systems of N. benthamiana. Vaccines manufactured by Kentucky Bioprocessing and Medicago are in clinical trial stages, while two vaccines and a therapeutic product are yet in their developmental pre-clinical stages [8]. iBio is developing a VLP-Based COVID-19 in the tobacco plant. Likewise, a group of scientists at the Queensland University of technology has thoroughly assessed the genome sequence of N. benthamiana. They are putting their efforts to utilize the plant's genome for the production of vaccines against COVID-19 [23]. Also, researchers at The University of California San Diego are combining advanced manufacturing strategies with molecular plant farming in which the Virus can explicitly infect legumes but not humans and is engineered in such a way that mimics SARS-CoV-2 to provoke the immune system [23].

Plant-based vaccines may also face challenges in their developments the same way as other vaccines do. To fulfill the requirements of regulatory agencies for approval, this technology needs to be validated based on their safety by employing large-scale clinical trials. Certain plant-made biopharmaceuticals have got approval to be used in humans and the conduction of clinical trials for plant-made influenza vaccines is encouraging. Owing to its low cost, rapid availability, and safe nature, a plant-made vaccine may revolutionize the field of vaccinology in the years to come.

4. TRANSGENESIS

It is the process by which Transgenic plants express foreign genes with industrial or pharmaceutical value [37]. Scientists and physicians are working to understand the new viruses and pathophysiology of the diseases to discover potential treatment regimes, effective therapeutic agents, and vaccines [38]. One effective method is to insert valuable genes from an entirely different species into a target plant, yielding a transgenic plant that acts as a factory for therapeutic products and emergency manufacturing of antiviral drugs, vaccines, and diagnostic reagents

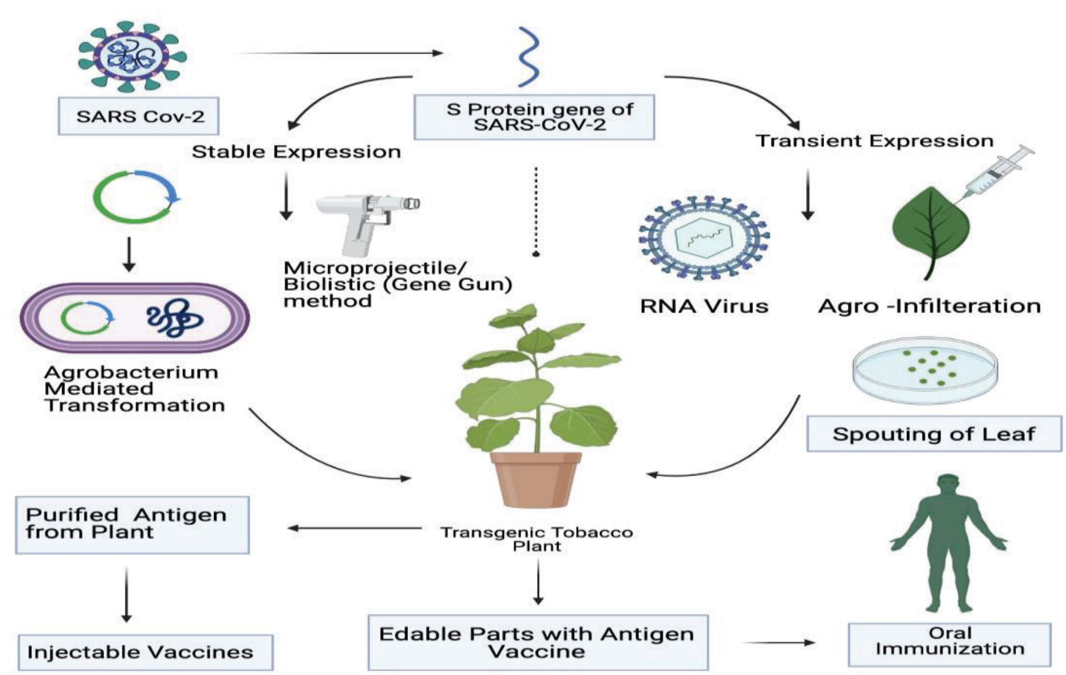

Fig. 2. Illustration of different strategies employed during pharming in plants potentially used to produce medicine against SARS-CoV-2 and other viruses

to reduce the spread of diseases and to save lives [12]. Different avenues where transgenesis in plants could help fight pandemics are given below (Figure 2).

4.1 Transgenic Secondary Metabolites against SARS-CoV-2

Plants are an essential source for numerous innovative bio-active compounds. A variety of different plant secondary metabolites (SM) are serving as vital drugs that exhibit extensive pharmaceutical and therapeutical properties with more minor side effects. To enhance the productivity and accumulation of target compounds transgenic plant cells can be manipulated in vitro [39]. To tackle future pandemics and COVID-19 caused by viruses, plant's secondary metabolites (PSMs) act as effective anti- SARS-CoV-2 molecules for further drug developmental processes and optimization [40].

Flavonols, a class of Flavonoids, can inhibit crucial proteins involved in an infective cycle of coronavirus. Flavonoids can inhibit SARS-CoV proteases, 3CLpro, PLpro, NTPase/helicase, and N protein of SARS-CoV. In fruits, a 72-fold increase in flavonol production was observed by chalcone isomerase gene overexpression from petunia in tomatoes. [41]. Glycyrrhizin obtained as an extract from licorice root is comprised of Glycyrrhetinic acid, Flavonoids, hydroxyl coumarins, and β-sitosterol that is spotted to have an essential anti-SARS-CoV activity [42].

Isoquinoline alkaloids like cepharanthine, tetrandrine, and fangchinoline can inhibit the expression of nucleocapsid and spike protein in SARS-CoV- OC43 in human lung cells. Such alkaloids can also intercalate DNA [43]. In berberine (isoquinoline alkaloid) biosynthesis, an enzyme (S)-scoreline 9-O-methyltransferase (SMT) is involved that controls the proportion of coptisine alkaloid: berberine and columbamine in cells of Coptis japonica. A 20% increase in enzyme activity was observed by this gene overexpression, which results in an increased level of columbamine and berberine from 79% in wild-type cells to 91% in transgenic cells. This study shows that an enzyme's overexpression in a pathway leads to increased flux of alkaloids which can be effective against COVID-19 and future pandemics [44].

4.2 Anti- SARS-CoV-2 MAbs Production in Plants through Transgenesis

Plant biotechnology gives a potential solution to the pandemic through the synthesis of affordable plant-made antibodies. In the case of passive immunization (antibody-mediated therapy), a great point is the cross-reactivity of the anti-SARS-CoV-1 Virus with SARS-CoV-2 Virus, proposing that the already produced biopharmaceuticals may well combat COVID-19. Hence the monoclonal antibodies (MAbs) are an effective therapeutic agent due to their potential for the COVID-19 treatment [11]. Researchers have discovered the capability of a plant expression system for manufacturing therapeutically appropriate human anti-SARS-CoV-2 MAbs like B38 and H4 that can be used as a diagnostic or therapeutic reagent. The MAbs can be expressed and assembled in Nicotiana benthamiana leaves using a geminiviral vector [45]. The B38 and H4 antibodies block the binding between the cellular receptor angiotensin-converting enzyme 2 (ACE2) and spike glycoprotein receptor-binding domain (RBD) of the Virus [46]. Researchers have further discovered the possibility of anti-SARS-CoV monoclonal antibody (MAbs) CR3022 and receptor-binding domain (RBD) of SARS-CoV-2 in N. benthamiana. The plant produced RBD showed specific binding to the SARS-CoV-2 receptor, angiotensin-converting enzyme 2 (ACE2) [47].

4.3 Vaccines Production through Transgenesis

Transient or stable expression of foreign genes results in the production of specific vaccines in plants. It has revealed that genes that encode antigens of viral and bacterial microbes can be expressed so that they hold their immunogenic properties [37]. The potential of plant expression systems for making vaccines against SARS-CoV-2 by expressing recombinant chimeric proteins, virus-like particles, sub-unit proteins, and other biologics are under study [48]. The expression of N protein in tobacco revealed that the injection and oral delivery of the N protein to the BALB-C mice increases the amount of IgG and IgA respectively in the experimental mice sera, representing the Anti-SARS-CoV activity of the vaccines [49].

By using transient expression systems, injectable vaccines can be produced that offer maximum protein yields and are now acquired at the industrial level to manufacture VLPs-vaccines and other biopharmaceuticals that help to provoke immunity against different antigens [11].

4.4 Production of Vitamins in Plants through Transgenesis against SARS-CoV-2

Several meta-analyses results have shown the significant benefits of a high dose of vitamin C injected intravenously (IV). Lactate secretion caused by the triggered immune cells can be inhibited by vitamin C treatment that possibly protects the innate immunity. This effect may help COVID-19 patients, as the SARS-CoV-2 usually affects the lower respiratory tract [50]. The vitamin C content can be enhanced two- to three folds in Arabidopsis thaliana by the overexpression of GalUR genes, this indicating the viability of engineering enhanced vitamin C levels in plants using this gene [51]. The level of vitamin E can also be increased by up to 4 fold in a transgenic Tobacco and A. Thaliana leaves by the plastidic expression

Table 1. Studies on the antiviral efficacy of several medicinal herbs against various coronavirus strains.

Coronavirus Strains	Plant species	References
SARS-CoV	*Lycoris radiate*	[75]
	Lindera aggregata	[76]
	Artemisia annua	
	Isatis indigotica	
	Pyrrosia lingua	
	Boenninghausenia sessilicarpa	[77]
	Lonicera japonica	[78]
	Eucalyptus spp.	
	Panax ginseng	
Bovine coronavirus (BCV)	*Amelanchier alnifolia*	[73]
	Cardamine angulata	
	Rosa nutkana	
	Verbascum Thapsus	
SARS-CoV (Hong Kong strain)	*Dioscorea batatas*	[79]
	Cassia tora	
	Taxillus Chinensis	
Ten different strains of SARS-CoV in fRhK4 cell line	Baicalin (*Scutellaria baicalensis*)	[80]
	Glycyrrhizin (*Glycyrrhiza uralensis*)	
HCoV-229E	Mulberry (*alba, Morus alba* var. *rosa*, *Morus alba* var. and *Morus rubra)*	[81]
	Calophyllum blancoi	[82]
	Pelargonium sidoides	[83]
SARS-CoV PLpro	*Psoralea corylifolia*	[84]
	Sambucus formosana	[85]
HCoV-NL63		
HCoV-OC43	*Stephania etrandra*	[86]
MERS-CoV EMC/2012	*Aglaia sp*	[87]

of the rat TATase gene [52].

5. GENE EDITING/GENOME EDITING IN PLANTS AGAINST PATHOGENS AND VIRUSES

Plants and pulses are the best sources of secondary metabolites, and they are known for the ability they show regarding human health [53]. Let's come to phytochemicals; those molecules which are synthesized in large amount by plants are known as phytochemicals [54]. Phytochemicals are non-nutritive substances present in massive amounts in plants [55]. Diet including fruits and vegetables can overcome the risk of various chronic diseases [56]. Also, phytochemicals can be used as a treatment for infections caused by bacteria and fungi [57]. It has been reported that there are multiple ways through which phytochemicals play a significant role in health; they can work as substrate, cofactors for an enzyme, and inhibitors. They can also work as a trap for toxic substances or fermentation substrate to multiple Bacteria and much more [58].

The success in the production of secondary metabolites by modifying or inserting new genes into in vitro culture has widened the chances of increasing or improving the production of phytochemicals [59]. This whole process took over three decades to demonstrate the feasibility of transformation in plants [60], which was the start of the genetic engineering era, and it compelled many countries for transgenic crops [59]. The availability of enough information regarding metabolic pathways made it easier for scientists to improve the quality and quantity of phytochemicals, leading to several successful experiments [61-64]. These plants were then used against many diseases, as scientists observed that various chronic diseases are inversely at risk to the food which contains antioxidant phytochemicals [65]. The success in improving and producing phytochemicals can be done by either qualitative or quantitative engineering approaches such as the engineering of β-carotene in rice grains [66, 67]. In rice, the elevation of iron content by two-fold increases [68] and enhances the ascorbic acid by seven-fold [69]. Moreover, the scientists also checked the activity of several plants against many pathogens. For example, mangrove plants were found very effective against many bacteria like Klebsiella pneumonia, Streptococcus pneumonia, Escherichia coli, and Enterococcus faecium [70].

Due to the unavailability of the vaccine and the treatment, Covid-19 caused high motility and morbidity. Scientists around the world tried different methods and techniques to overcome the pandemic. Such as Hyperbaric oxygen therapy (HBOT) [71], Packed red blood cell transfusions [72], Chloroquine/hydroxychloroquine treatments [73], and secondary metabolite; phytochemicals have a significant role in human health, a number of scientists were using different plants against the Virus. Such as the Indian Ayurvedic herb, Asparagus racemosus (Wiled). Through docking analysis, they used Asparoside-C, Asparoside-D, and Asparoside –F against SARS-CoV-2, and by their docking score and affinity, they confirmed that there is a receptor-binding domain on two proteins of SARS-CoV-2 viz. NSP15 Endoribonuclease and spike and were found that they are both effective against these proteins. [74].

Different experiments observed the antiviral potential of multiple medicinal compounds or phytochemicals against various strains of coronavirus (CoV), which are described in Table 1. Different Phytochemicals have different mechanisms against corona virus-like, Rosa nutkana, and Amelanchier alnifoliain habit or deduct the activity of enteric coronavirus [73], Torreya nucifera (Amentoflavone) inhibit the nsP13 helicase and 3CL protease, other plants like Black tea, also known as TheaFlavin is effective against SARS-CoV and inhibit its 3C-like protease [88].

6. CONCLUSION AND FUTURE PROSPECTS

Although treatment and vaccines for COVID-19 are available, we must continue the improvement of the treatment for a better antiviral effect. We should be cost-effective having more minor side effects while making an antiviral drug against particular viral proteins or gene the main problem we face is that while replicating the Virus continuously mutate itself, as studied in HIV and HSV and Hepatitis-B Virus. However, natural resources play an essential role in the development of new antivirals.

7. ACKNOWLEDGMENTS

The authors acknowledge the role of the Department of Biotechnology, University of Malakand, in providing necessary resources and environment.

8. DECLARATION

We confirm that the manuscript contains original secondary data and is not published nor under consideration elsewhere. Moreover, the consent of all authors has been obtained.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 31-35 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)658

Pakistan Academy of Sciences

Review Article

Biosafety and Biosecurity Measures in Clinical/Research Laboratory: Assistance with International Guidelines

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Abstract: Nowadays, laboratory biosafety and biosecurity are serious global issues in clinical laboratories and academic research labs. With the high rate of biological harm caused by evolving infectious diseases and bioterrorism, we all are equally responsible for biosafety and biosecurity at the workplace, but it is the main responsibility of governments from all over the world that must raise their awareness and preparedness for detecting and containing hazardous biological agents, this responsibility includes not only providing a biological safe environment for laboratory workers and also biosafety of others in the institution and community. Safe and effective laboratory operations and pathogen handling determine the safety of the laboratory workers, pathogens, and the laboratory environment, these all are the important variables in the successful performance of laboratory assays. The basic concepts of laboratory management, laboratory biosafety, and laboratory biosecurity are addressed in this article. In addition, DURC (dual-use research of concern) is the research that is commenced for lawful reasons that produce knowledge, information, technology, and/or products that can be used for both good and bad aspects. Biosafety and biosecurity measures should be included in the laboratory policy manuals for the guidance of laboratory personnel.

Keywords: Biosafety, Biosecurity, Research/Clinical Laboratory, Biorisk, Biosafety Levels, DURC

1. INTRODUCTION

Biosafety and biosecurity guidelines are the set of policies, standards, and procedures, these are essential for lab personnel who deal with bacteria, viruses, parasites, and fungi. These guidelines are intended to ensure that biosafety and biosecurity policies and procedures are properly managed and regulated at all levels of lab management [1]. Laboratory-acquired infection (LAI) is very common in the world, as underlying reports of LAI showed at the beginning of the 20th century, almost 4,000 LAIs occurred in the year from 1930 to 1978 globally [2]. Many biosafety and biosecurity challenges are encountered in laboratories around the world, and that need to be addressed the proper guidelines of biorisk management for lab workers. The World Health Organization (WHO) has proposed an agent categorization for laboratory use that divides the into four risk groups based on these characteristics, inherent factors, and the mode of transmission. The four groups cover the hazards to both the laboratory staff and the community [3]. In addition, Biosecurity is defined by the World Health Organization as the containment, principles, technology, and practices used to prevent the deliberate abuse or release of microorganisms. It is based on four primary controls are physical, personal, material and information [4]. Life sciences research is meant to be beneficial but could easily be misapplied to cause harm. Biosafety and biosecurity have become now essential elements for laboratories.

Received: March 2021; Accepted: July 2021
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2. LABORATORY BIOSAFETY/ BIOSECURITY MANAGEMENT

It is important to follow the training and guidelines for Identification and controlling the hazardous condition in the lab, and eliminating or reducing the risks of infectious agents for the protection of the lab staff from bio-hazardous material.

2.1 Sample Receiving

Extremely infectious biological specimen such as blood, tissues, body fluids and microbiological culture carries certain infections. These are collected from fields, collection points, and sample collection areas in different healthcare facilities. General guidelines for sample receiving include the following: (1) Check proper labeling (patient's ID/name, date of collection, biohazard label). (2) Receiving Samples with proper personal protective equipment (PPEs). (3) Check Sample condition (Temperature, Volume/amount, biohazard tag). (4) Check sample container or device. The sample will be rejected if; there is any leakage occurred in the sample container [3, 5]

2.2 Sample Processing

Facilities of the clinical & research laboratories are defined as basic-biosafety levels (BSL) 1 to 4. The infectious material is characterized in Risk of four Groups (RGs) based on relevant risk to laboratory workers and the community Table 1 lists the descriptions of the WHO and NIH risk group classifications. This allows all lab personnel to deal with all samples and pathogens responsibly and harmlessly. Samples processing should be performed according to their risk factor of BSL level [5].

3. LAB-BIOSAFETY

A Biosafety plan is a bunch of preventive measures intended to decrease the danger of bio-hazard exposures and reduce the risk of laboratory accidents. A biosafety plan including the group of related components covers lab exercise, primary barrier (personal protective equipment (PPE), biosafety cabinets, and Mechanical Pipetting Devices), and the secondary barrier, such as facility design features, engineering control, facility design, separation of the lab from public access, hand washing stations and standard operating procedures (SOPs). Implemented biosafety programs in the lab should be protecting the lab workers and their families from laboratory-acquired infections, save the environment from contaiminants and maintain the quality of natural environment.

The fruitful biosafety strategy exists on the arrangement of the biosafety committee. The Biosafety committee's role is the execution of the risk assessments, guaranteeing, execution, requirement, and setting up the biosafety levels. Additionally, the committee makes the policy of risk assessment, risk characterization, risk evaluation,

Table 1. Description of the World Health Organization (WHO) and US National Institutes of Health (NIH) risk groups [3, 5].

Risk Groups (RG)	Individual Risk	Community Risk	Descriptions
1st Risk Group (RG1)	Low	Low	Agents that have not been linked to disease in healthy people or animals.
2nd Risk Group (RG2)	Moderate	Low	Agents are linked to comparatively rare diseases, but preventive or treatments are frequently available.
3rd Risk Group (RG3)	High	Low/Moderate	Agents linked to severe human diseases for which there could be treatment and prevention measures (high individual risk but low community risk)
4th Risk Group (RG4)	High	High	Agents that are likely to cause significant or severe human disease for which there are no easily available preventative or therapeutic measures (high individual risk and high community risk)

and risk mitigation.

Biosafety is everybody's duty—the institutional supervisors, the biosafety official(s), the lab administrators, lab technologists/researchers, and other lab supporting staff (housekeeping).

Biosafety policy/program should be made by the upper management, scientists, safety coordinators, security officers, etc [3 , 5 , 7-10].

3.1 Biosafety Level (BSL)

Biosafety levels (BSL) are used to determine which protections are required in a laboratory setting to protect personnel, the environment, and the general public.

3.1.1 Biosafety Level-1 (BSL-1)

BSL-1 involves infectious material or poisons unknown cause infection in a healthy person.

3.1.2 Biosafety Level-2 (BSL-2)

BSL-2 builds on BSL-1, which involves agents causing a moderate risk to personnel, the community, and the environment. For processes with high aerosol potential, work is performed in the Biological Safety Cabinet.

3.1.3 Biosafety Level-3 (BSL-3)

BSL-3 laboratory has special engineering and design features under negative pressure with directional airflow and differential pressure. Only authorized personnel have access to the facility. It involves dealing with biological agents that have the potential to cause serious or deadly diseases by inhalation or other routes. Everything must be handled in a Class II Biological Safety Cabinet.

3.1.4 Biosafety Level-4 (BSL-4)

BSL-4 deals with extremely dangerous and contagious pathogens. Infection from those pathogens is untreatable [4].

4. PERSONAL PROTECTIVE EQUIPMENT (PPE)

Personal protective equipment (PPE) is used in clinical/research laboratories; in this context, PPE includes gloves, surgical masks, gowns, goggles, or a face shield, as well as for special procedures, respirators (N95) [6].

5. BIOLOGICAL SAFETY CABINET (BSC)

BSC has laminar airflow and HEPA (high-efficiency particulate air) filtration. It has designed to contain aerosols produced during work with infectious materials.

5.1 Basic Guidelines for Working in BSC

- Plan work and proceed conscientiously.
- Users must check the certification, expiry date, and other parameters before starting work.
- Do not obstruct the BSC's defensive airflow pattern.
- Until beginning work in the BSC, double-check that the lab doors are locked.
- Make sure the BSC has been running for at least three minutes before beginning work.
- Keep material storage in and around the BSC to a minimum.
- When the cabinet is in operation, restrict traffic in the room.
- Wipe work surfaces with 70% alcohol or any other chemical as per sectional SOPs.
- Wipe each item you need for your procedures with disinfectant before placing it in the cabinet [7].

6. LABORATORY WASTE MANAGEMENT

The organization must ensure that a policy is in place to reduce the amount of clinical waste generated, as well as a good waste management strategy for the natural environment and toxins. Laboratory waste is segregated on-site based on the method for treatment and disposal. Infectious solid and sharp waste is disposed of by incineration while liquid waste is disinfected in kill tanks & neutralization tanks. Non-infectious waste should be in a green bag. Contaminated/ potentially infectious materials except sharps should be in a red bag. Sharps will be disposed of in a sharps container or a danger bin [7-10].

7. SPILL MANAGEMENT

In case of a biological and chemical spill in the lab, before starting to clean up a spill, everyone involved in the cleanup must assess the extent of the spill and follow the proper cleanup protocol. (1) Take a deep breath, warn others, exit the room, and shut the door. (2) Show the sign for everyone to see (Spill clean-up in progress). (3) Reach the area to begin cleanup after 30 minutes, when the aerosols have settled. (4) Get a spill-control pad (Spill Kit). (5) Put on the proper protective equipment (gloves, gowns, safety goggles, footwear). (6) Pick up any broken glass with forceps or heavy gloves and place it in a sharps bag. (7) Use a disposable absorbent material to cover the spill. (8)Discard infected material into a red autoclave bag after it has absorbed the oil. (9) Clean the area as if it were a small spill. (10) Inform the supervisors/assistants (11) Record the spill on the Incident Reporting Form as well as the monthly Safety Indicators [10-11].

8. LAB-BIOSECURITY

Biosecurity in the lab refers to a collection of protective measures aimed at reducing the possibility of purposeful removal (robbery) of biological material. Nowadays, the world faces new challenges in ensuring public health safety and security in the term of possible domestic and foreign terrorism including the use of harmful biological agents or toxins. The pathogenicity or transmissibility of potential pandemic infections has generated biosafety and biosecurity issues, including potential dual-use risk linked with the exploitation of such research's information or results. [7,12]. A biosecurity strategy addresses the threat that intentional exploitation or the arrival of a natural specialism poses to human and animal health, the environment, and the economy. Such an arrangement incorporates an organization of interrelated components.

8.1 Actual/Physical Security

The facility's physical protection includes access control, a security camera system, and an intrusion warning system. Unauthorized individuals are not permitted to access the lab without prior authorization.

8.2 Personnel Security

Staff security incorporates exceptional status (screening, personality confirmation, instructive/proficient accreditation confirmation, military assistance confirmation, public criminal checks, and monetary checks) and security interviews.

8.3 Checking/Controlling/Accountability of Materials

Material control and accountability tend to the insider danger and directly implies the staff that works with infectious pathogens and poisons that could be appealing to bioterrorism. Accountability expects coordination, correspondence among materials and persons along with maintaining the records of inventory, pathogens List, equipment, and data record (hard/soft copy).

8.4 Transportation-Protection

The transportation of biological/infectious material outside of an enclosed area, such as an inspection, general safety or symptomatic testing center, or a vaccination development center is referred to as transport protection. The vehicle may be traveling across international borders, within a region, or a similar office. Moving biological material requires (1) ensuring that the prerequisites are met. For transportation (material exchange arrangements, approved beneficiaries, guidelines); (2) preparing the distribution (grouping, bundling, stamping, marking, documents and parcel delivery to the courier) (3) dispatching the board (material exchange arrangements, authorized beneficiaries, guidelines, approved receipt confirmation, the records, and access controls).

8.5 Digital Information Security

Data security suggests to issues the passwords for personal/official computers and laptops.

8.6 Camera Monitoring

A 24 hours camera monitoring facility should be available for the surveillance of illegal activities [12-16].

9. CONCLUSION & RECOMMENDATIONS

It is necessary to increase the knowledge with the requirement of BSL-1 to BSL-4, research/

clinical lab administration, lab biosafety, and lab biosecurity measures. An approach to deliver biosafety, biosecurity, and the DURC concepts in the laboratory through conduct the lectures series in institutes for life sciences faculties/students/researchers.

Laboratory biosafety and biosecurity measures are the essential elements of clinical and research laboratories, and their risks should all be considered carefully. Laboratory management, laboratory biosafety measures, and laboratory biosecurity guidelines are all used to protect laboratory personnel, the environment, the product, and the community. The importance of laboratory biosafety and laboratory biosecurity policies must be emphasized. One method to deal with this is to introduce the concept through education, in both life sciences and engineering departments.

DURC's mission is to preserve the benefits of life sciences research while reducing the risk of misusing the knowledge, information, goods, or technologies developed because of such research. Finally, since biosafety and biosecurity are not constrained by national borders, significant debate, and exchange of ideas at the levels of the scientific community, international organizations, and countries should be required to develop a suitable governance mechanism at the international level to prevent the exploitation and abuse of research/pathogens, as well as to mitigate large-scale biological integrity loss, with a focus on both ecological and human health.

10. ACKNOWLEDGEMENTS

We would also like to thank Furqan Kabir, Lead Trainer, Biosafety, Biosecurity, Biorisk Management, Aga Khan University for sharing his knowledge in this regard.

11. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 37-43 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)653

Pakistan Academy of Sciences

Research Article

Impact of Climate on Confirmed Cases of COVID-19 in Lahore: A Predictive Model

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Abstract: World over, the weather conditions are usually categorized as predictors of respiratory viral infections. This study uses a stepwise linear regression model to explore the effects of climate factors or weather factors such as temperature, humidity, wind speed on the spread of COVID-19 in Lahore city (Pakistan). The study was conducted in Lahore College for Women University, Lahore, and the data regarding the cases of COVID-19 in the Lahore district was obtained from the Primary and Secondary Health Care Department Punjab, from 18th March 2020 to 25th August 2020 while the weather statistics were obtained from Environmental Protection Department, Lahore. A predictive model by regression was designed in which day-to-day humidity, wind speed, average temperature, and their impact on confirmed cases of COVID-19 in Lahore were analyzed. The independent variables in the model were average temperature (C), humidity (%), wind (km/h) and the dependent variable was the number of daily established cases of COVID in Lahore. The result of the analysis shows the effectiveness of the proposed model and the impact of climate parameters on the assessment model. The study illustrates that the above model can be used to predict the future spread of COVID-19 based on the above-mentioned climate factors. As such, it proves as a useful modality to predict new cases for the government and other health agencies.

Keywords: COVID-19, climate, climate factors, stepwise linear regression, Lahore.

1. INTRODUCTION

The COVID-19 is an ongoing epidemic, caused by a coronavirus (nominated as severe acute respiratory syndrome coronavirus-2-SARS-CoV2) has been identified in December 2019 in Wuhan City, China. By rapidly spreading in too many countries, this virus has created a universal threat and was acknowledged as a "public health crisis of international significance" by the World Health Organization (WHO) on 30th January 2020. This virus spread from one person to another via indirect and direct physical contact with contaminated objects or surfaces besides open places with aerosols and aerial droplets generated through speaking, coughing, or sneezing [1, 2].

Numerous studies have been conducted in different countries to monitor this pandemic to strengthen their understanding of the factors that affect viral transmission (including climate and weather). Respiratory viral infectious diseases like corona-virus, Severe Acute Respiratory Syndrome (SARS) and seasonal influenza tends to increase in the colder environment, thereby weather conditions are considered as one of the factors for acquiring respiratory infections [3-6].

Although seasonal influenza and SARS have some similarities to the new coronavirus, it has been hypothesized that its existence may depend on the weather and assumed that low temperature tends to promote the escalation of the virus [6,7]. In comparison, humid and hot weather has been speculated to minimize viral activity [8]. This remains uncertain as there is no consensus to date, on the effect of humidity and temperature to regulate COVID-19 dynamics or tropical-humid environments overwhelm its transmission. Globally, several studies investigated the outbreak of COVID-19 in terms of different weather conditions, especially humidity and temperature [9,11]. These studies have described negative or positive associations among the transmission of COVID-19

Received: March 2021; Accepted: July 2021
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and changes in humidity and temperature. This study aims to discover the consequence of climatic conditions on the prevalence of COVID-19 in Lahore (Pakistan) and to propose a predictive model to improve the prediction.

2. MATERIALS AND METHODS

2.1 Data Set

The data regarding established cases of COVID-19 in the Lahore district were obtained from the Primary and Secondary Health Care Department, Punjab, for the period from 18th March 2020 to 25th August 2020. The data of climatic or weather parameters were obtained from the Environmental Protection Department, Lahore. As the levels of humidity, temperature, and wind speed change during the day, the average temperature, humidity, and wind speed were noted each day for the period from 18th March 2020 to 25th August 2020. The MATLAB software was used to form a predictive model to determine the role of climate on the outburst or spread of COVID-19. A regression model was used for this purpose. Data was analyzed to finalize which predictors should be used in the final regression model. This process was referred to as the variable selection problem. Two objectives were involved in determining these subsets of independent variables. The first objective was to make the model thorough and as precise as possible i.e., to include every regressor that was even slightly related to the dependent variable. Simultaneously, the least number of independent variables were included in the model because irrelative predictors make the model imprecise, complex thereby making the processor of variable selection a conundrum: striking a balance between fit (as many predictors as possible) and simplicity (to include a minimum number of regressors).

2.2 Screening of Candidate Variables for the Model

When a large group of variables occurs, the candidate variables for use in the Model may be screened from the group of variables by using the techniques namely, the Backward (Step-Down) Selection Procedure, or the Forward (Step-up) Selection Procedure [12, 13]. The Backward (Step-Down) Selection method is less popular because it begins with a model in which all candidate variables have been included. However, because it works its way down instead of up, it always retaining a greater value of R-Squared. The problem is that the models selected by this mode may contain variables that are not required. The user sets the effect level upon which variables can pass in the model. The backward selection model begins with variables used for all candidates in the model. At every phase, the least important variable is removed until no non-significant variables remain. The significance level at which variables can be removed from the model is set by the user.

The Forward (Step-up) Selection Technique is frequently used to provide a preliminary screening of the candidate variables when a large group of variables occurs [12]. A reasonable approach would be to use the forward selection procedure to obtain the best ten to fifteen variables and then apply the all-possible algorithm to the variables in this subset. This procedure is also a good choice when multicollinearity is a problem. The forward selection method is simple to define. It begins with no candidate variables in the model. A variable that has the highest R-Squared is selected. At each step, only that candidate variable is selected in which R-Squared raises the most. When none of the remaining variables are major then the process of adding variables is stopped. This exercise of adding variables in the model is practiced by taking into account the fact that once a variable enters the model, it cannot be deleted.

P-value is the measure to judge the result of the model. P-value is essentially the probability of having results that are almost at the extreme as the actual results, assuming the null hypothesis is correct.

F-test is done to check if the group of the independent variable are collectively significant or not. F-value shows whether a regression model gives better-fitted data than a model having no independent variable. Similarly, the p-value and F-value are the measures used to check the effectiveness of the constructed model. The smaller values mean that extreme outcomes are highly unlikely to occur.

2.3 Stepwise Regression

Stepwise regression is a logical mode for removing and adding parameters from a generalized linear or linear model based upon the statistical importance in elucidation of the response variable. The technique initiates with a primary model and then relies on the descriptive power of incrementally smaller and larger models. The stepwise regression uses backward and forwards regression to determine the last model. At every phase, the function pursuits for terms to remove from the model or add to the model based on the p-value for the F-test of the change in the sum of squared error that results from adding or removing the term. Stepwise regression takes the following steps: The initial model was fitted.

1. Examine a set of available terms not in the model. If any of the terms have p-values less than an entrance tolerance (i.e. if it is unlikely a term would have a zero coefficient is added to the model), add the term with the smallest p-value and repeat this step; otherwise, go to step 3.
2. If any of the available terms in the model have p-values greater than an exit tolerance (that is, the hypothesis of a zero coefficient cannot be rejected), remove the term with the largest p-value and return to step 2; otherwise, end the process.

3. RESULTS

In the present study, weather factors (average wind speed, average temperature, and average humidity of each day) and COVID -19 data of each day for the period from March 2020 till August 2020 were used. Figures 1, 2 & 3 showed the number of COVID-19 cases with average temperature (^{o}C), average humidity (%), and average wind speed (km/h) respectively in the Lahore district. In the regression model, the independent variable was Average Temperature (^{o}C), Humidity (%), Wind (km/h), and the dependent variable was daily confirmed cases of COVID in Lahore.

Linear regression model: COVID-CASES ~ 1 + Humidity + Wind*Temperature

The constructed linear regression model was presented in Table 1. ANOVA test was performed on the constructed model which gave the results as presented in Table. 2. The values of R^2 were at the appropriate level, and the regression model was significant since the final model p-value is 0.02361 which is less than 0.05. The regression model plot as shown in Figure 4 illustrates that the model was significant because a horizontal line does not fit between the confidence bounds, which is consistent with the p-value obtained. The residuals plot of the linear regression model is shown in Figure 5.

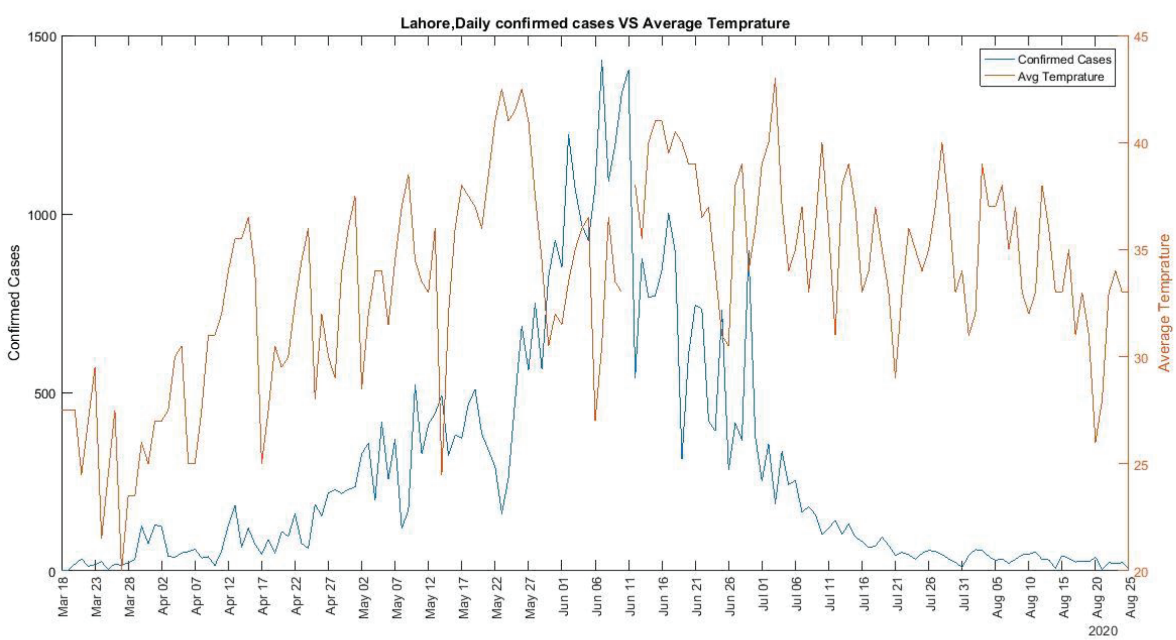

Fig. 1. The average temperature during the study period in Lahore

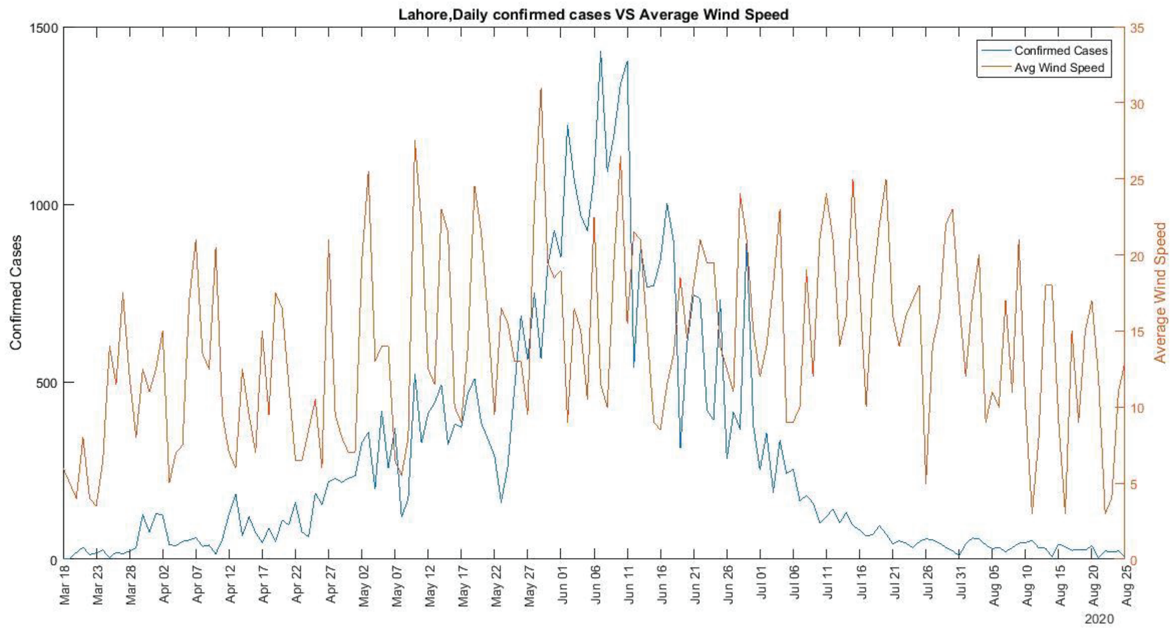

Fig. 2. The average wind speed during the study period in Lahore.

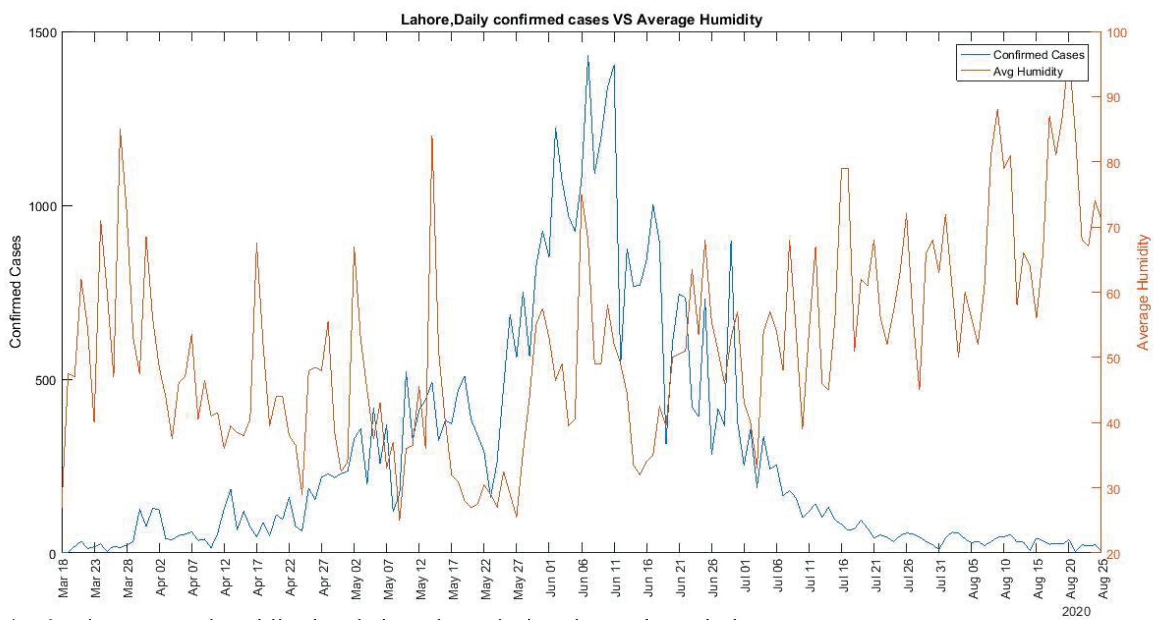

Fig. 3. The average humidity levels in Lahore during the study period.

Table 1. Linear Regression Model Estimated Coefficients:

	Estimate	SE	T Stat	P -Value
(Intercept)	-1082.8	495.57	-2.1849	0.031
Wind	91.921	34.082	2.6971	0.007
Humidity	-4.5781	1.7713	-2.5847	0.011
Temperature	41.669	14.477	2.8784	0.005
Wind: Temperature	-2.3094	1.0103	-2.2858	0.024

R-squared: 0.187, Adjusted R-Squared 0.166

F-statistic vs. constant model: 8.91, p-value = 1.67e-06

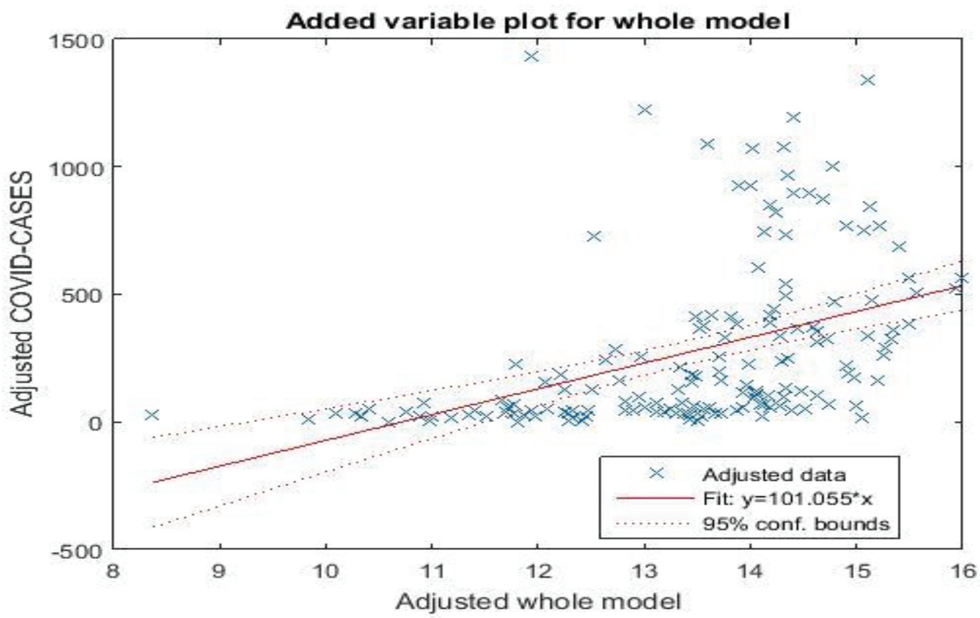

Fig. 4. Regression model plot between adjusted climate parameters and COVID cases

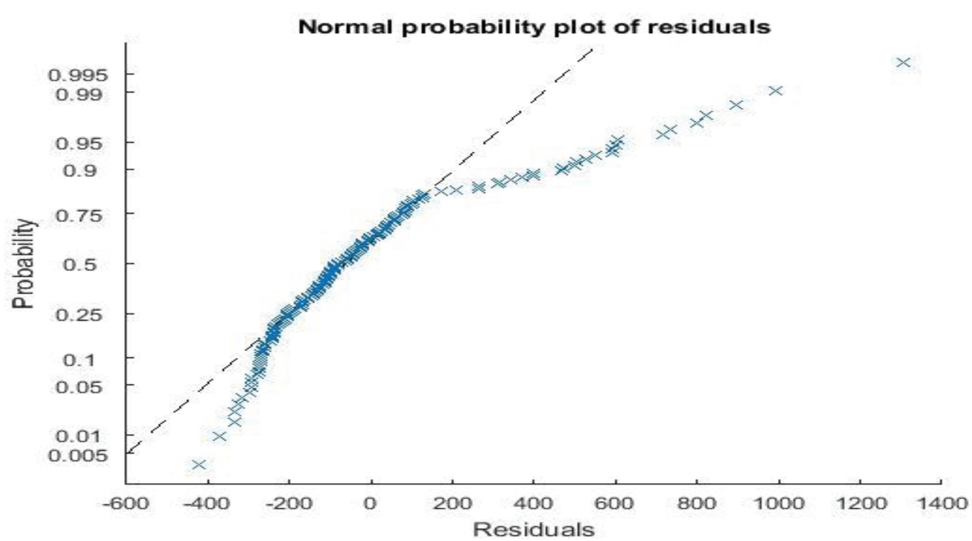

Fig. 5. Residuals of Linear regression.

Table 2. ANOVA Analysis

	Sum Sq	D F	Mean Sq	F	P- Value
Wind	1.1463e+06	1	1.1463e+06	13.004	0.001
Humidity	5.8885e+05	1	5.8885e+05	6.6804	0.011
Temperature	3.3949e+05	1	3.3949e+05	3.8515	0.051
Wind : Temperature	4.6057e+05	1	4.6057e+05	5.225	0.024
Error	1.3663e+07	155	88146		

4. DISCUSSION

In China's 130 cities (except Wuhan), it was reported that in the initial stage of the epidemic, the spread of COVID-19 was due to humidity and temperature [9]. In the same way, the facts of other countries, excluding China revealed that everyday COVID-19 new cases were inversely related to temperature and relative humidity [9], while the mean day to day temperature had a major effect on the daily reported COVID-19 cases. For the spreading of SARS-CoV-2, the optimum temperature was 8.07 °C and humidity varied from 60% to ~90%. This is in accordance with Wang et al [8] that who emphasized the role of temperature and reported that 8.72°C was the optimum temperature for viral spread. The decrease of temperature and humidity tends to increase the virus survival and spread while the study by Rouen *et al*.,(2020) recorded a negative relationship between the daily temperature and prevalence of COVID-19 [14], a study by Rasheed *et al*., (2020) reveal that a climatic aspect, especially temperature, is a noteworthy environmental issue for the dispersion of COVID-19 infection [15]. Reportedly, COVID-19 infection was primarily more associated with temperature between 5°C to 11°C and absolute humidity levels less than three [10].

A positive association between COVID-19 prevalence and increases in temperature and humidity has also been documented [3, 16, 17]. In Singapore, positive associations with the amount of regular as well as aggregate COVID-19 cases were related to absolute humidity and temperature [3]. A direct association was observed during the preliminary stage of the COVID-19 outburst and ambient temperature. The mainstream of the reviewed studies indicates a stimulating trend. In several studies, the important negative association of COVID-19 cases with humidity and temperature suggests that SARS-CoV-2 has a periodic impact, with dry and cold circumstances, which can promote the spread of the new coronavirus, while at higher temperatures and higher comparative humidity levels, the virus loses its capability and thus weakens its spread [17, 18]. The results were consistent with reports of SARS-CoV, influenza, and other corona-viruses, i.e., Extreme Acute Respiratory Syndrome [19]. The occurrence of COVID-19 may, therefore, verify to be seasonal, which presents a warning in the winter season for increased transmission. Certain studies have also revealed that COVID-19's physical distribution has been confined to countries with colder climates so far [20]. In the low-temperature area of China, this virus first appeared in early winter, and subsequently, the main epidemic happened in further Asian states such as Middle East, Europe, Japan, and South Korea where during January and February 2020, the temperatures were far below 0°C [21, 22]. As the virus spread, a narrow climatic band was initially grouped into new epicenters. Cold weather tends to be further conducive to the transmission of COVID-19 that elucidate the consistently higher COVID-19 cases in these countries.

5. CONCLUSION

This study indicates the use of a stepwise linear regression model to check the effects of climate factors such as temperature, humidity, wind speed on the spread of COVID-19. The model is evaluated based on p-value, Root mean square value, and R squared value. The results show that the above model can be used to predict the future outbreak of COVID-19 based upon the above-mentioned climatic factors. It could prove useful to the primary and secondary health care departments, clinical/hospital management, as well as pandemic prevention-related agencies of the Government in preventing the virus. The study can be further extended by using the other regression models and deep learning methods.

6. ACKNOWLEDGEMENTS

The authors acknowledge the cooperation by the Primary and Secondary Health Care department as well as of Environmental Protection Department, Punjab.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 45-54 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)654

Pakistan Academy of Sciences

Research Article

Impact of SARS-CoV2 Treatment on Development of Sensorineural Hearing Loss

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Abstract: Ototoxicity had been a known viral manifestation. This raised the possibility for the current pandemic caused by the novel coronavirus to induce temporary or permanent auditory manifestations. The struggle to search for therapeutic options to counter the pandemic is still underway. Meanwhile, the management had relied on using antimalarials and antivirals; which themselves have proven ototoxic repercussions. Interestingly this brings to a point of further debate whether the auditory dysfunction is induced by the virus alone or by the drugs that are used to pacify the pathology of the viral exhibition or both. This article will channel this current implication of the hearing loss debate focusing on the mainstay regimen for SARS-CoV-2 management. A bibliographic search was performed to review current literature in scientific databases PubMed, Research Gate, and Google Scholar. Published articles encompassed within our inclusion criteria were reviewed thoroughly to draw possible outcomes. Reported SARS-CoV-2 manifestations are sensorineural hearing loss with disturbed vestibulo-auditory symptoms. Reviewed research data suggested aggravation in ototoxicity induced by these medications. This upsurges the controversies surrounding the safety and efficacy of the medications currently in active use for managing SARS-CoV-2 infection. Further therapeutic strategies need to be researched for equipping the arsenal to effectively treating SARS-CoV-2 and its complications.

Keywords: SARS-CoV-2, COVID-19, toxicity, sensorineural hearing loss, antimalarials, anti-virals

1. INTRODUCTION

Hearing loss following viral infection has been reiterated for years. Viral-induced hearing loss is known to have mild to profound impact, being either unilateral or bilateral and conductive or sensorineural in type [1]. The patient presentations differ as per the type of viral infection and the pathology that follows; be it direct or indirect damage to the anatomical structures of the inner ear or means to activation of the host immune system to inflict damage to the hearing apparatus [2]. Of note here is the ototoxic potential of medications that are being used to counter the viral pandemic at hand [3].

The outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, along with its predecessors [SARS-CoV-1 and Middle East respiratory syndrome (MERS)], have had life-threatening consequences [4]. The SARS-CoV-2 symptoms may appear as early as 2 days or may extend up to 14 days after viral exposure [5]. Olfactory and gustatory symptoms are the key findings of the SARS-CoV-2 virus apart from respiratory, cardiologic, and GIT symptomatology [6]. Current literature also hints at neurotrophic and neuroinvasive features with SARS-CoV-2 infection [7]. By extension, the role of SARS-CoV-2 as a causative agent in hearing loss has recently been noted and has ignited researchers to investigate and collate updated evidence available on the prevalence of hearing loss in SARS-CoV-2 infected patients.

Finding this possible link between SARS-CoV-2 to hearing loss has become relevant as to what medicament plan do we offer to counter

Received: March 2021; Accepted: July 2021
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SARS-CoV-2; would it predispose the individual to further the hearing loss impairment? Currently, multiple off-label anti-virals and antimalarials such as lopinavir-ritonavir, hydroxychloroquine, chloroquine, and certain other drugs are used in preference for the early treatment of SARS-CoV-2 [8]. Prescribing medications with possible targets against the SARS-CoV-2 virus, having a lack of pharmacokinetic and pharmacodynamic implications was similar to shooting in the dark, which resulted in several unwanted consequences that added further dilemma to the pandemic scenario. Lack of therapeutic options against SARS-CoV-2 could be identified from the fact that even with randomized control trials pointing the insignificant effect of antimalarial drug-like hydroxychloroquine on death and recovery rate in SARS-CoV-2; along with other antimalarial and antiviral drugs are still under consideration in health care centers across the world [9-11]. One of the unwanted consequences is ototoxicity which interestingly had been a shared adverse effect to most of the available therapeutic arsenal against SARS-CoV-2; including antibacterial, anti-inflammatory, antimalarials, anti-virals, and certain immunomodulatory compounds [12, 13].

Ototoxicity is defined as hearing impairment, tinnitus, and imbalance caused by damage to the inner ear structure and vestibular system which could result in a temporary and/or permanent hearing disability [14]. Chloroquine and hydroxychloroquine had previously been sought for the findings of auditory dysfunction such as sudden sensorineural hearing loss, vertigo, and tinnitus in patients treated with these anti-malarial drugs [11, 15]. In chronic cases, the symptoms got worse and irreversible based on how long the therapy continues [16]. In some clinical settings, anti-malarial drugs were prescribed in combination with an antibiotic like Azithromycin, to increase the therapeutic effectiveness in SARS-CoV-2 patients. Unfortunately, the combination of drugs could potentiate mild to severe audiological manifestations based on the duration of exposure [17].

Hearing loss has detrimental effects on quality of life, depending on the age of onset as it impinges on cognitive skills, learning abilities, and results in an invisible handicap of the affected person with severe psychological solitary confinement [18]. Tinnitus increases the risk of anxiety and depression. In addition, inner ear pathology leading to spatial disorientation increases the chances of physical injury and also leads to poor productivity with social isolation [19-21]. The hearing loss still does not have standard care and has limited pharmacologic modalities [15]. The purpose behind this study is to collate the impact of SARS-CoV-2 and the currently used medications; to signify the ototoxic impact that could be generated with a charged focus on neurologic inferences such as sensorineural hearing loss (SNHL). This would help direct our attention towards risk mitigation and limiting strategies for current SARS-CoV-2 therapeutics to avert unwanted outcomes.

2. MATERIALS AND METHODS

The systematic literature search was performed primarily on three databases: PubMed, Research Gate, and Google Scholar engines on 24th February 2021 to identify pertinent literature to our study question, as presented in Figure 1. The search strategy was based on the MeSH words: COVID-19, SARS-CoV-2, ototoxicity, tinnitus, hearing loss, antiviral and antimalarial medications.

Automated database search provided 59 articles with an addition of another research paper by bibliographic hand search, cumulating to a total of 60 articles. Repeated articles were removed manually. Studies that focused on therapeutic implications of SARS-CoV-2 along with ototoxic complications formed the basis of our inclusion criteria and 21 papers were identified for further screening for inclusion in our systematic review. These research articles were based on different study categories i.e. clinical reports, case studies, case reports, and cross-sectional studies. Studies with insufficient findings, inconclusive data, and non-English publications were excluded from our article. On thoroughly reviewing the filtered papers, key findings were extracted for further analysis.

3. RESULTS AND DISCUSSION

Steering the therapeutic realm of coronavirus SARS-CoV-2 infection is currently reliant on symptomatic treatment and supportive care. The development and issuance of vaccines are a time-consuming and

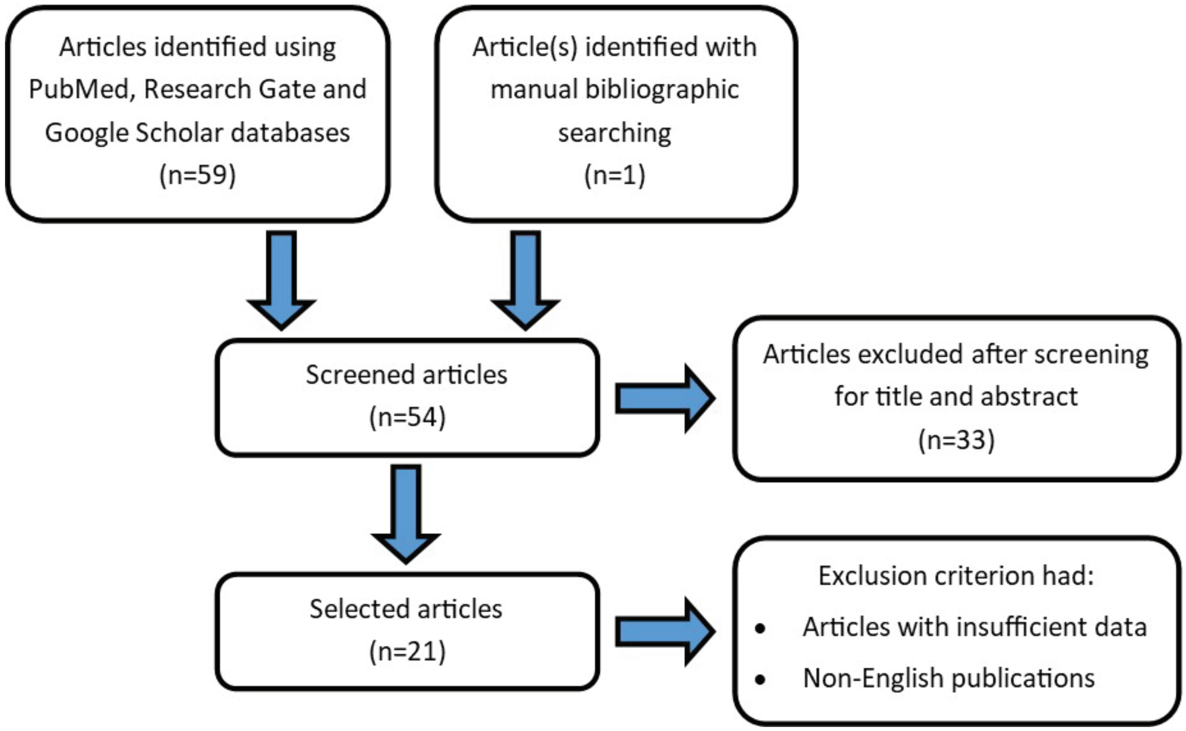

Fig. 1. Flow diagram of the study selection process.

expensive modality, which is currently underway. To curtail the spread of the infection, the logical introduction of antiviral medications along with repurposed anti-malarial drugs was maintained, as the initial management strategy. Even though these medications had previously been studied for their pharmacotherapeutic influence, but their safety profile under the current coronavirus pandemic still needed further clinical evidence. A comprehensive list of drugs, summarized in table 1, was compiled including antimalarials, anti-virals, antibiotics, immunomodulatory agent, and their combinations. One of the enthusiastic approaches taken to manage SARS-CoV-2 was to prescribe antimalarials - chloroquine and hydroxychloroquine. Since limited therapies are available for possible SARS-CoV-2 management. A study on chloroquine revealed that it might block SARS-CoV-2 viral replication by elevating endosomal pH followed by a decrease in viral load; identifying antimalarials to be in vogue [23]. Interestingly, randomized control trials on hydroxychloroquine had been published that showed no impact on improving SARS-CoV-2 infection mortality [22]. This confronted the FDA-approved Emergency Use Authorization (EUA) of chloroquine and hydroxychloroquine, which had been placed during the early days of the pandemic. However, the agency reversed its approval due to new information obtained from clinical trials that reported confounding results. Various studies reported adverse impacts on the heart, ears, skin, eyes, and muscles in patients treated with chloroquine and hydroxychloroquine [24-27]. FDA issued a warning in April 2020 regarding hydroxychloroquine use in non-research and off-label health care settings due to these emergency safety concerns (1).

The scarcity of available pharmacologic options still upholds the consideration of antimalarials for therapy. This trend engages the ototoxic impact of chloroquine and hydroxychloroquine use that could include reversible and irreversible symptoms of tinnitus, SNHL, and vertigo. Various studies revealed either atypical audiograms or loss of hearing ability in SARS-CoV-2 positive patients treated with chloroquine. Some reported cases had short-term SNHL after chloroquine treatment while this condition was improved after cessation of treatment [28, 29]. Dwivedi and his coworker

Table 1. List of currently used therapeutics for SARS CoV-2 with related ototoxic implications.

Category	Medication	Type of Hearing Loss	Drug Effect	Other Symptoms	Range of Hearing Loss	Laterality	References
Anti-Malarial	Chloroquine	SNHL	Both	Tinnitus and Vertigo	Severe	Bilateral	[69-71]
	Hydroxychloroquine	SNHL	Both	Tinnitus	Mild to Severe	-	[72, 73]
Anti-Viral	Lopinavir–Ritonavir	-	Reversible	-	Moderate	Bilateral	[74, 75]
	Ribavirin	SNHL	Both	Tinnitus	Severe	Unilateral	[47, 49, 76]
	Ivermectin (also anti-parasitic)		Reversible	Vertigo and Dizziness	-	-	[49]
Non-Steroidal Anti-Inflammatory Drugs	Aspirin	-	Reversible	Tinnitus and Vertigo	-	Bilateral	[77-80]
	Indomethacin	-	Reversible	Tinnitus and Vertigo	-	-	[77, 80]
	Naproxen	-	Irreversible	-	-	-	[80]
	Ibuprofen	-	Irreversible	Tinnitus and Vertigo	-	-	[77, 80]
Antibacterial	Azithromycin	SNHL	Both	Tinnitus and Vertigo	Mild to Severe	Bilateral	[14, 81]
Immunomodulators	Interferons (IFNs)	SNHL	Reversible	Tinnitus	-	Bilateral	[49, 62, 63]

*SNHL= Senserineural Hearing Loss

reported a case of 52 years old male experiencing bilateral permanent deafness followed by blurring of vision and vertigo after a single dose of chloroquine [30]. In another study, 13 out of 70 patients treated with chloroquine experienced reversible cochlear injury despite normal tone audiogram outcomes detected by brainstem audiometry [31]. Likewise, permanent severe SNHL cases have also been reported [30-32]. Similar to chloroquine, hydroxychloroquine treatment also produced ototoxicity in reported cases. Reversible SNHL was observed after hydroxychloroquine administration along with irreversible cases [33-36]. Tinnitus is also described together with loss of hearing in a few cases [34, 35].

The SNHL or tinnitus was observed to manifest much earlier after chloroquine use rather than hydroxychloroquine where prolonged administration was recognized. The recommended chloroquine dose for SARS-CoV-2 patients is 1g for 10 days that is extensively higher than the acclaimed dose for treatment of malaria (1g for 3 days) [37]. Furthermore, the suggested hydroxychloroquine dose for SARS-CoV-2 patients is 800 mg, which is later reduced to 400 mg for 4 days [38]. Another study recommends giving hydroxychloroquine at 600mg for 10 days. Although proposed doses of hydroxychloroquine and chloroquine for SARS-CoV-2 patients are considerably higher than suggested for malarial patients, similarly duration of use also influences the possible development of side effects. Even with numerous reports on chloroquine and hydroxychloroquine-induced hearing loss are established and FDA aims to restrict their use; very limited research exists on SARS-CoV-2 patients, permitting clinical reluctance on restraint [39].

The antivirals considered for managing SARS-CoV-2 include antiretrovirals and non-antiretrovirals. The antiretrovirals include Nucleoside Reverse-Transcriptase Inhibitors (NRTI) and Protease inhibitors. These drugs may develop adverse outcomes with different frequencies and intensity based on the active cellular mechanism being involved such as NRTI causes mitochondrial toxicity that leads to hearing impairment [40]. In one Australian longitudinal study, a positive association of NRTI treatment disposing to hearing loss has been evidenced in HIV-positive patients. Another study reported a positive association between hearing impairment and mitochondrial dysfunction with protease inhibitor therapies in a 44 years old male patient [41]. As per available literature, the duration of anti-retroviral therapy could predispose to hearing impairment while some studies did not find any adverse effects on hearing loss after using the therapy for a longer duration [42].

The protease inhibitors such as lopinavir alone and/or in combination with ritonavir have proven in-vitro inhibitory activity against SARS-CoV-1, SARS-CoV-2, and MERS [43]. This had been a widely prescribed drug combination for the treatment of SARS-CoV-2 based on in-vitro experiments, preclinical cases, and observational reports. Lopinavir is the main component in the inhibition of SARS-CoV-2 target protein and along with ritonavir; it potentiates the efficacy of lopinavir by elevating its half-life. Lopinavir–ritonavir inhibits the highly conserved protease of SARS-CoV-2; thereby inhibiting viral replication in the host body and decreases the viral load. It has proven neurotoxic effects which results in bilateral SNHL with depressive symptoms when used for extended periods. Hearing reverts back to normal following discontinuation of the drug regimen [44].

Ribavirin targets viral replication by inhibiting viral mRNA synthesis. This antiviral agent is used in combination with interferons for SARS-CoV-2 treatment [45]. Apart from its significant therapeutic potential in SARS-CoV-2 treatment it also holds proven literature on ribavirin-induced ototoxicity. A recent study has shown a severe sudden hearing loss in patients who received combined therapy of ribavirin and interferons, with a reversible and irreversible impact [46-49]. Due to the scarcity of research data, it is still unknown that either ribavirin is the sole reason behind sensorineural hearing loss or is it due to the effect of combining multiple drugs [50].

Ivermectin is a broad-spectrum anti-parasitic drug that has also shown to actively inhibit viral replication in SARS-CoV-2 patients [51]. Although very limited studies are available on ivermectin-induced ototoxicity the available data has suggested vestibuloauditory manifestations of ivermectin therapy [52]. It is used alone and also in combination with other drugs like ribavirin, chloroquine, and hydroxychloroquine [53].

The use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin was directed to reduce the inflammatory condition from developing, in addition to dealing with headache and possible body ache. Aspirin can induce reversible ototoxicity and causes high-frequency tinnitus. It has been noted to produce a temporary effect on sensory cells of the inner ear, whilst the pathogenesis is still not clear [54]. Other NSAIDs such as ibuprofen and naproxen produce irreversible hearing impairment but have a lower incidence rate as compared to aspirin [55].

Azithromycin is widely used to treat bacterial infection and the human influenza virus [56, 57]. It is used in combination with hydroxychloroquine and lopinavir-ritonavir; as a three-drug combination with greater efficacy [58]. This triple therapy was observed to be very effective as lopinavir-ritonavir with azithromycin elevates blood serum levels of hydroxychloroquine, effectively targeting the virus but also predisposes to ototoxicity [59].

Interferons (IFNs) are signaling proteins such as interferon-α (IFN-α) and interferon-β (IFN-β), with antiviral and immunomodulatory efficiencies [58]. Research on clinical data with COVID-positive patients reveals IFN-α therapy to clear the viral load by decreasing inflammatory biomarkers [60, 61]. Nevertheless, both in-vivo and in-vitro studies have confirmed the ototoxic impact of IFNs therapy. In one study, participants who were on IFN-α or IFN-β therapy; 35% later developed SNHL (18/49 patients) and 29% developed tinnitus (14/49 patients) [61]. In another study, 37% of total study patients documented SNHL when treated with IFNs [62]. Hearing loss associated with IFNs therapy is reverted to normal upon discontinuation of therapy within two weeks [63]. Animal models suggested cochlear damage but the exact mechanism of IFNs induced hearing loss is still not clear [64].

Therapeutics of SARS-CoV-2 involves a combination of drugs, and recent research data supports the proposition of synergistic audiovestibular function when multiple ototoxic SARS-CoV-2 drugs are co-administered for the treatment purpose [15]. Currently, the most commonly presented combinations are hydroxychloroquine with lopinavir-ritonavir or IFNs, azithromycin, and ivermectin, lopinavir-ritonavir, IFNs, and ribavirin. There is insufficient data available that could point out the possible mechanistic of ototoxic synergism of these therapies [59]. In addition, several compounding factors also exist that increases the chance of acquiring this induced ototoxicity such as age, underlying impaired hearing function, hereditary component, and impaired drug elimination function [65]. Kidney function is suspected to affect up to 20% to 40% in SARS-CoV-2 patients and results in a disturbing drug elimination process [66]. It will lead to the elevated serum ototoxic therapeutics level of drugs like hydroxychloroquine, chloroquine, and ribavirin [65]. In aged individuals, as the renal elimination system deteriorated with time so they are more prone to develop ototoxicity when exposed to these medications [67]. Similarly, individuals with underlying hearing disability or having a positive family history of hearing loss are at a higher susceptibility to develop ototoxic complications when they use these drug combinations [68].

Clinical improvisation is needed to screen the serum level of these potential ototoxic therapeutics in people at risk including high-risk populations to overcome the lifelong consequences of these drugs on hearing and balance functioning [65].

4. CONCLUSION

The SARS-CoV-2 pandemic has added a lot of pressure over the therapeutic domain to challenge the evolving nature of SARS-CoV-2 viral infection. Available research data on SARS-CoV-2 therapeutics have shown symptomatic improvement with raised possibility of ototoxic impact with these proposed therapeutic interventions. With an active rise in SARS-CoV-2 cases, patients unknowingly were exposed to these ototoxic medications. It is therefore of utmost importance that the healthcare services should vigilantly assess the symptoms of hearing impairment, tinnitus, and vertigo; to prevent lifelong health consequences. Further research needs to be directed to search for alternate medications which would help mitigate the viral infection and would thereby avert the ototoxic impact of currently used therapies and help prevent long-term disability.

5. ACKNOWLEDGEMENTS

TG and MZY planned and structured the initial draft. All the authors took part in writing and approving the article (HR, AK, KG, MZY, and TG. MZY reviewed the final draft of the manuscript. The author(s) would want to acknowledge the Pakistan Academy of Sciences for the publication of the manuscript. There is no exchange of financial support for research and authorship for this article.

6. CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 55-67 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)655

Pakistan Academy of Sciences

Research Article

Showcasing the Internationally Prioritized Medicinal Plants to Counteract the Pandemics – Potential Remedies for COVID-19 and other Forms of SARS

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Abstract: Indigenous communities throughout the globe respond to COVID-19 by their traditional medicinal systems as primary health care. Our lab was part of an international study that discusses the outcomes of a rapid response, preliminary survey during the first phase of the pandemic among social and community contacts in five metropolises heavily affected by the COVID-19 health crisis (Wuhan, Milan, Madrid, New York, and Rio de Janeiro) and in twelve rural areas or countries initially less affected by the pandemic (Appalachia, Jamaica, Bolivia, Romania, Belarus, Lithuania, Poland, Georgia, Turkey, Pakistan, Cambodia, and South Africa). Primarily, people have relied on teas and spices ("food-medicines") to prevent and mitigate its symptoms. Urban diasporas and rural households seem to have repurposed homemade plant-based remedies that they use on daily basis to treat the flu and other respiratory problems and hence consider among the healthy foods. The most remarkable shift in many areas has been increased in the consumption of ginger and garlic, followed by onion, turmeric, lemon, chamomile, black tea, nettle, chili pepper, and apple. This study serves as a baseline for future systematic ethnobotanical studies countering COVID-19 and other vicious types of viruses. It aims to inspire in-depth research on how use patterns of plant-based foods and beverages, both "traditional" and "new," are changing during and after the COVID-19 pandemic. Our reflections in this study call attention to the importance of ethnobiology, ethnomedicine, and ethno-gastronomy research into domestic health care strategies for improving community health. Some of these economically important plants are suggested to be extensively analyzed experimentally, for active ingredients, phytochemicals, and the precursor of vaccines and probable remedy of SARS including COVID-19.

Keywords: Ethno-medicines; COVID-19; Pandemic; Potential Remedies.

1. INTRODUCTION

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; COVID-19) in the city of Wuhan China in 2019 and its ubiquitous nature spread it worldwide and caused millions of deaths so far [1, 2]. On the 30th of January 2020, World Health Organization (WHO) declared an emergency of international concern for public health because of the SARS-CoV-2 [3]. Close contact with infected individuals is the main reason for the speedy propagation of COVID-19 [4, 5]. Facial protection, social distancing, increased hygiene, and avoiding large gatherings are various measures to flatten the spread of the COVID-19 pandemic [6, 7], but these nonmedicinal precautions are inadequate for long-term management [8]. Researchers are working hard for the development of vaccines, nanomedicines, and other effective medications to prevent the fatal COVID-19 [9, 10]. Local communities throughout the globe use various homemade medicines to treat the coronavirus disease at indigenous levels [8]. The traditional societies living in villages are mostly economically marginalized, but they are wealthy in terms of biological resources [11]. They use homemade herbal medicines and natural

Received: March 2021; Accepted: July 2021
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products for decades to prevent viral diseases [12] which shows great potential and efficiency. In the current era, it is undeniable that natural products and herbal medicine are still playing a crucial role in drug discovery [13, 14].

Plant species used as antiviral remedies have acceptable toxicity which makes it a potential prophylactic candidate to combat the SARS-COVID-19. *Magnolia officinalis* bark a combination of TCM and Western treatments is being used extensively in China to treat COVID-19 patients [15]. A study has screened 125 Chinese herbal medicines with the potential of direct inhibition on COVID-19. More than 125 species of plants are ingredients in these treatment formulations. These generally include *Glycyrrhiza* spp., *Panax* spp, and *Cibotium barometz*. Species used in more than five formulations are *Glycyrrhiza* spp., *Magnolia officinalis*, *Scutellaria baicalensis, Ephedra* spp., *Atractylodes macrocephala, Forsythia suspensa, Pogostemon cablin.* The main species found in the commercial trade of liquorice are *Glycyrrhiza glabra* and *Glycyrrhiza uralensis* [7].

Frequently used plants in SARS treatments include *Lonicera japonica*, *Glycyrrhiza uralensis, Astragalus membranaceus, Atractylodes macrocephala, Angelica sinensis, Scutellaria baicalensis, Schisandra chinensis,* and *Panax quinquefolius* [16]. Keeping in mind the importance of homemade medicines we have based our study on a recommendation proposed by [17]. They have mentioned the 10 most important plant species widely used across 17 case studies. We have gathered literature on these plant species for a respiratory disorders, viral disease in general, and COVID 19 in particular. This research aims to showcase the highly important plant species for taming and management of the pandemic days.

2. MATERIALS AND METHODS

The data generated by reviewing the current state of the knowledge and research articles on the perception and the use of medicinal plants as potential remedies against COVID-19. Relevant research articles were searched via different search engines such as "google scholar", ISI web of knowledge, PubMed, without any restriction of the publication year using different combinations of search terms; medicinal plants OR ethnomedicinal plant and (against COVID-19 and respiratory problems), phytochemical OR phytoconstituents, remedies OR recipes, globe OR worldwide. Through initial selection, 186 research articles were appraised addressing medicinal plants with antimicrobial properties. Further screening of these research articles by reading abstract, conclusion (results and discussion where needed) overall 124 published articles met the aim of the study.

3. RESULTS AND DISCUSSION

Plants have great therapeutic potential to cure various health disorders. Therefore, used all over the world against various diseases including viral infections. These are actively used in the form of herbal remedies and are preferred to encounter different diseases due to easy access, low cost, and least or no side effects. However, in this period of the unexpected viral outbreak with no possible treatment, people responded to COVID-19 preliminary with easily accessible plant species. Globally ten selected plants are reviewed for antiviral activity along with their action against respiratory disorders. These include common household plants i.e., *Zingiber officinale* Roscoe, *Allium sativum* L., *Allium cepa* L., *Curcuma longa* L., *Citrus limon* (L.) Osbeck., *Matricaria chamomilla* L., *Camellia sinensis* (L.) Kuntze., *Urtica dioica* L., *Capsicum annuum* L., and *Malus domestica* Borkh as shown in Table 1 and Figure 1.

3.1 *Zingiber officinale* Roscoe.

Zingiber officinale Roscoe, (Figure 1a) has been traditionally used for throat infections and broadly reported against many diseases. Many of the studies proved its activity against viral diseases in the form of potential extracts or other additives. According to [18], fresh ginger has an anti-viral activity that decreases plaque formation induced by the human respiratory syncytial virus (HRSV). Mishra et al., [19] reviewed that its extracts are involved in the modulation of inductive genes responsible for chronic inflammation while ginger's rhizome isolates such as ingenol and 6-shogaol exhibit antiviral activity. According to these studies, *Z. officinale* was a highly cited plant species used against COVID-19 as shown in Figure 2. Relative importance value highlights the ten most important

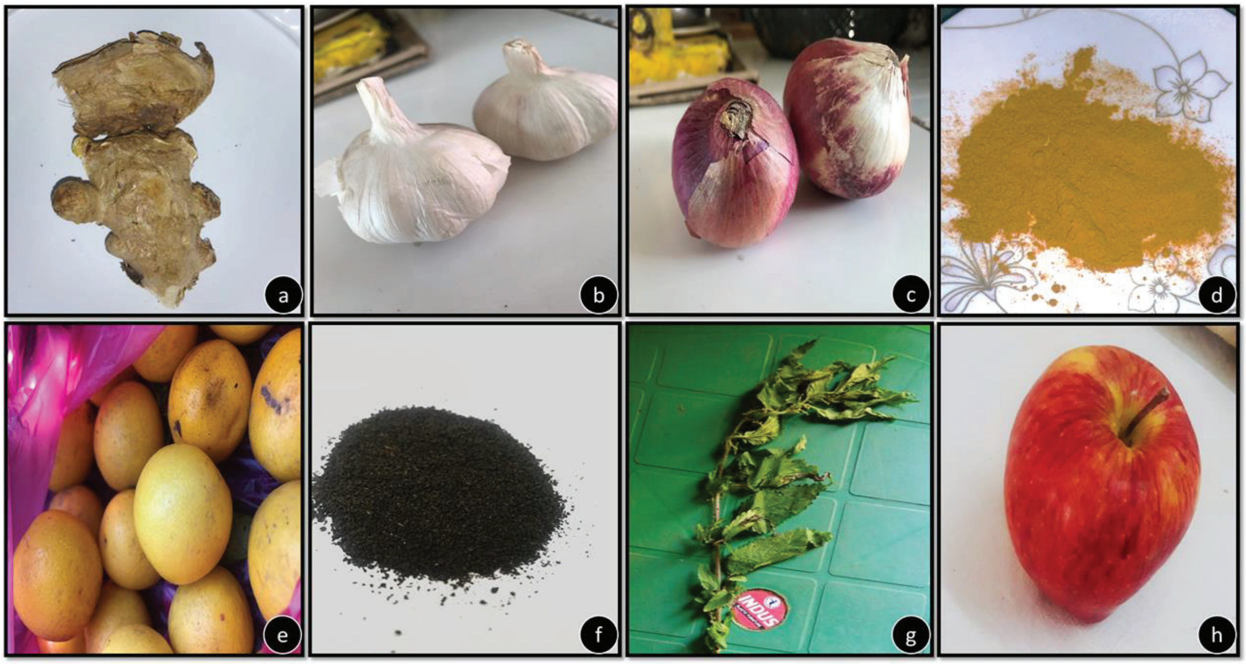

Fig. 1. (a) Ginger (b) Garlic (c) Onion (d) Turmeric (e) Lemon (f) Black tea (g) Nettle (h) Apple

Table 1. Top ten globally prioritized medicinal plants exhibiting anti-viral and respiratory therapeutic activities

S. No.	Plant Species	Anti-microbial (anti-viral)	Respiratory Diseases
1.	*Zingiber officinale* Roscoe.	[42-46]	[42-44, 47, 48].
2.	*Allium sativum* L.	[49-53]	[49, 51, 54-56]
3.	*Allium cepa* L.	[57-61]	[62-66]
4.	*Curcuma longa* L.	[67-71]	[71-75]
5.	*Citrus limon* (L.) Osbeck	[76-80]	[81-85]
6.	*Matricaria chamomilla* L.	[86-91]	[88, 92, 93].
7.	*Camellia sinensis* (L.) Kuntze	[94-99]	[100-102]
8.	*Urtica dioica* L.	[103-109]	[108, 110, 111]
9.	*Capsicum annuum* L,	[112-116]	[112, 117-119]
10.	*Malus domestica* Borkh.	[120-123]	[123,124]

taxa used among which ginger was the most significant of all, used as home remedies during the preliminary stage of pandemic (Figure 3). Ginger rhizome is the most vital part of the plant and is highly being used as a spice in cuisines, due to its high potential against throat infection dried or fresh ginger tea is the best remedy for its cure. Some, of the global evaluation of *Zingiber* showing anti-viral potential and therapeutic activity against respiratory disorders, are mentioned in Table 1. However, reported gingerol and zingerol extracted from ginger as active inhibitors of COVID-19 strain. Pakistan cost 71 million US$ in 2017 for importing 79,000 tonnes of ginger (https://pc.gov.pk/uploads/report/Ginger Cluster_Report.pdf). Our neighboring country is the world-leading country having similar climatic conditions to our Punjab province. If we identify suitable zones in our country for ginger production, we can save such a huge amount.

3.2 *Allium sativum* L.

Our study reveals *Allium sativum* (Figure 1b) as the second most abundantly (3.8 RI value) used medical plant against COVID-19 around the globe. Sulphur-containing phytoconstituents such as allicin, alliin, vinjldithiins, ajoenes, and flavonoids

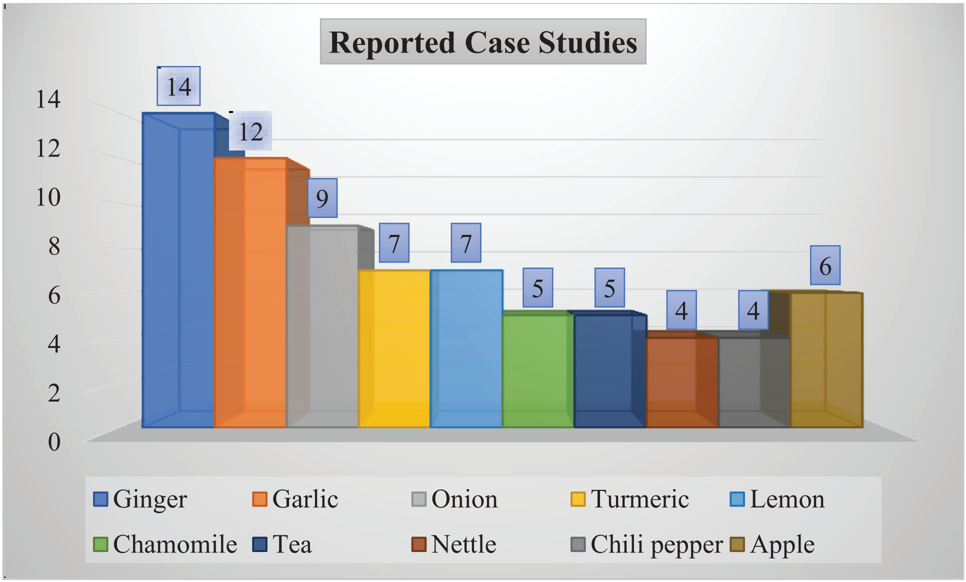

Fig. 2. Plant species reportedly used for the treatment of COVID-19

particularly quercetin have proven antimicrobial, antifungal, and antiviral properties [20]. Organic sulphur compounds have proven antiviral activities to human, animal, and plant viruses [21]. In Pakistan garlic is cultivated over 7973 hectares area in 2015 with a 72987 tonnes production [22]. Garlic is a very potential and economic crop and plays an important role in the livelihood of agricultural societies of the country.

3.3 *Allium cepa* L.

Allium cepa comes next to *A. sativum* against COVID-19 with a 3.7 RI value and is a rich source of polyphenols, flavonoids, saponins, organic sulphur along with other secondary metabolites [23]. Onion (ure 1c) is an economic and commercially important vegetable crop throughout the world. In Pakistan, it is cultivated over an area of 131.40 hectares. In 2018, the annual production of onion was 1.8 million tonnes (DOI: 10.13140/RG.2.2.11139.04647).

3.4 *Curcuma longa* L.

Curcuma longa is among the premier spices used in a variety of dishes due to its posed medicinal properties with a 2.9 RI value. Phytochemical profiling of turmeric seems to be dominated by curcumin and cyclocurcumin with anti-viral properties mainly by binding the active site of SRS CoV-2 protease [24 , 25]. Turmeric (Figure 1d) is an important figure of condiments and spices throughout the country. It is cultivated in different areas of the country, but the district Kasur is the leading district with an 80% share of the annual production of 30569 metric tonnes [26]. The demand for turmeric consumption increases with time due to which its per kg price also increases. Therefore, its cultivation on broad scales is crucial for the development of the livelihood of agricultural societies.

3.5 *Citrus limon* (L.) Osbeck.

Citrus limon is a medicinally very important plant with a 2.7 RI value widely used in different preparations. Its photochemistry reveals the presence of phenolic acids such as synaptic, ferulic, and p-hydroxybenzoic acid and phenolic compounds such as limocitrin, diosmin, hesperidin making it suitable against various microbial diseases [27]. Citrus (Figure 1e) is grown in different areas of the country. The total citrus production from 2014-2015 was 2.4 million tonnes [28]. The trend for Citrus gardens development increases in the region which is considered an important step for the employment of traditional societies.

3.6 *Matricaria chamomilla* L.

Chamomile is the single ingredient most popular herbal tea prepared from dried and brewed flowers for multiple medicinal purposes. Potential medicinal properties of *Matricaria chamomilla* accounted as out-turn of bioactive compounds. Phenolic compounds are the premier constituent of flowers essentially flavonoids such as quercetin, apigenin, luteolin, patuletin, and glucosides. Whereas terpenoids α-bisabolol and azulenes to chamazulene are ruling components of its essential oil [29]. The existing fact, of phytoconstituents occurrence, made chamomile tea a suitable alternative against respiratory and viral diseases. Chamomile is a popular medicinal herb used at large scales as an herbal tea in the region. Another species Matricaria recuitata is grown in the highlands for commercial purposes. Nowadays *Matricaria chamomilla* is grown in herbal medicines industries to produce homeopathic medicines, through the cultivation of this important species we can increase livelihood and employment chances in the region.

3.7 *Camellia sinensis* (L.) Kuntze

On the top of herbal teas comes green tea (*Camellia sinenses*) where dried leaves and flowers are used. *Camellia sinenses* (Figure 1f) brought to play against viral and respiratory diseases essentially due to diversified chemical compounds. Primarily polyphenolic constituents such as catechins, epicatechin gallate (ECG), epigallocatechin gallate (EGCG), and epigallocatechin (EGC) reported from previously studied literature of [30] against influenza virus by restricting or inhibiting replication and could be a possible reason for its recommendation against a broad spectrum of viral diseases. Polyphenol epigallocatechin-3-gallate found premier chemical compound against hepatitis B virus [31] and anti-HIV therapy. Pakistan imports tea from other countries like Kenya, Bangladesh, Brazil, etc. The government of Pakistan in 1986 established a national tea research station at Shinkiyari district Mansehra. A tea garden was established over 30 acres of land to enhance tea production in the country [32]. Unfortunately, tea production is still a challenge for Pakistan.

3.8 *Urtica dioica* L.

The stinging nettle possesses an Immune-modulatory effect that helped native people in symptomatic cure of COVID-19. Almost the same number of case studies were reported as that of Capsicum but, *Urtica dioica* L. (Figure 1g) is relatively much important than chilli and Apple having RI value greater than that of *Capsicum annuum* L. and *Malus domestica* Borkh as illustrated in Figure 3 & Figure 4. Nettle help in covid-19 mortality as reported by [33], 2020. It reduces pain and has anti-inflammatory therapeutic activity and can be used in the form of tea and food [34]. Leaf of *Urtica dioica* L. was used effectively as an anti-asthmatic agent and against lung diseases worldwide and initially at hospitals to relieve fever pain due to viral infection [35] (Table. 1). *Urtica dioica* is widely distributed in the temperate zones of both hemispheres. In Pakistan, the species is distributed in the Himalayan and few regions of the Karakoram and Hindukush mountainous ranges.

3.9 *Capsicum annuum* L.

Among the ten most potential plant species used in the primary phase of the pandemic, *Capsicum annuum* L. was also one of them. It is a common plant used globally as a spice in most cuisines. Its bio-active compounds are used to target the COVID-19 main protease (Mpro) but before it was utilized in the form of remedies to overcome the symptoms of this novel virus [36]. The global studies reported Capsicum as one of the widely used plants with an RI value of 1.2, in the first phase of the pandemic. It has been experimentally reported to possess antimicrobial i.e., anti-viral, anti-inflammatory, and effectively used against respiratory disorders (Table .1). The capsicum fruit is the food additive as well as traditionally it has been used to combat several disorders. Capsaicin is the most active alkaloid present in pepper that exhibits the binding affinity of COVID-10 protease [37] that made it capable of combating the pandemic. Sindh province of Pakistan is very popular for chilli production. The country contributes 2 lac tons of chillies. Unfortunately, with the increase in climatic changes, chillies production decreases in the region [38]. The introduction of suitable and potential varieties can lead to more significance in chilli production in the region.

3.10 *Malus domestica* Borkh.

Malus domestica (Figure 1h) is also documented as an important plant against antimicrobial activities. It contains a rich content of polyphenols both in its pulp and peels comparatively in more concentration

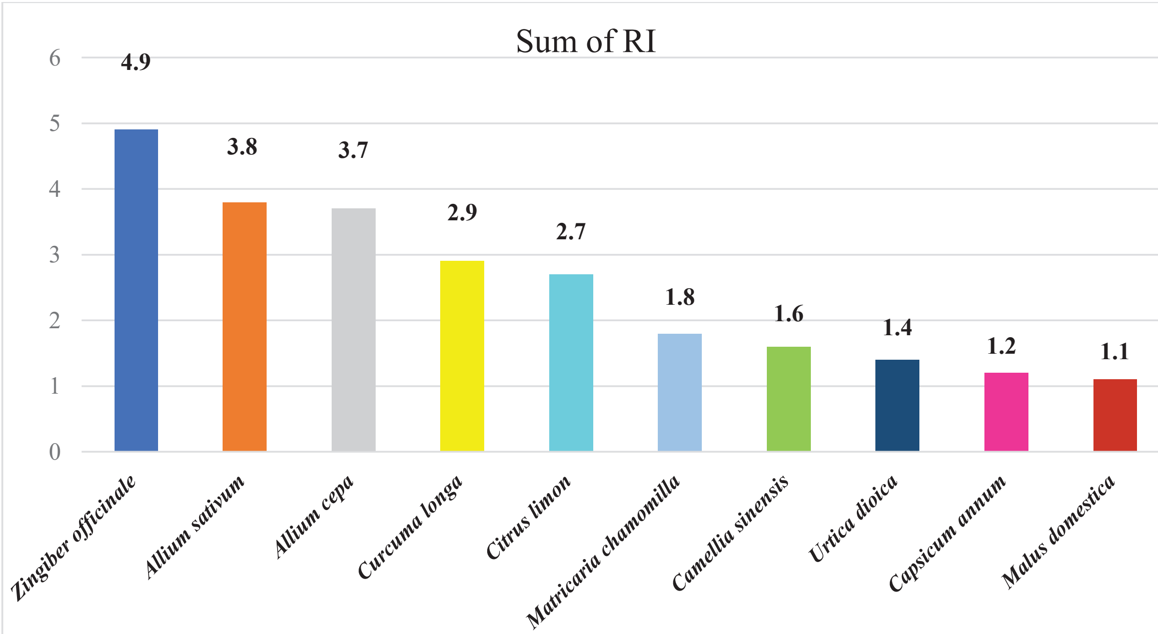

Fig. 3. Sum of the Relative importance of potential plants used during a pandemic

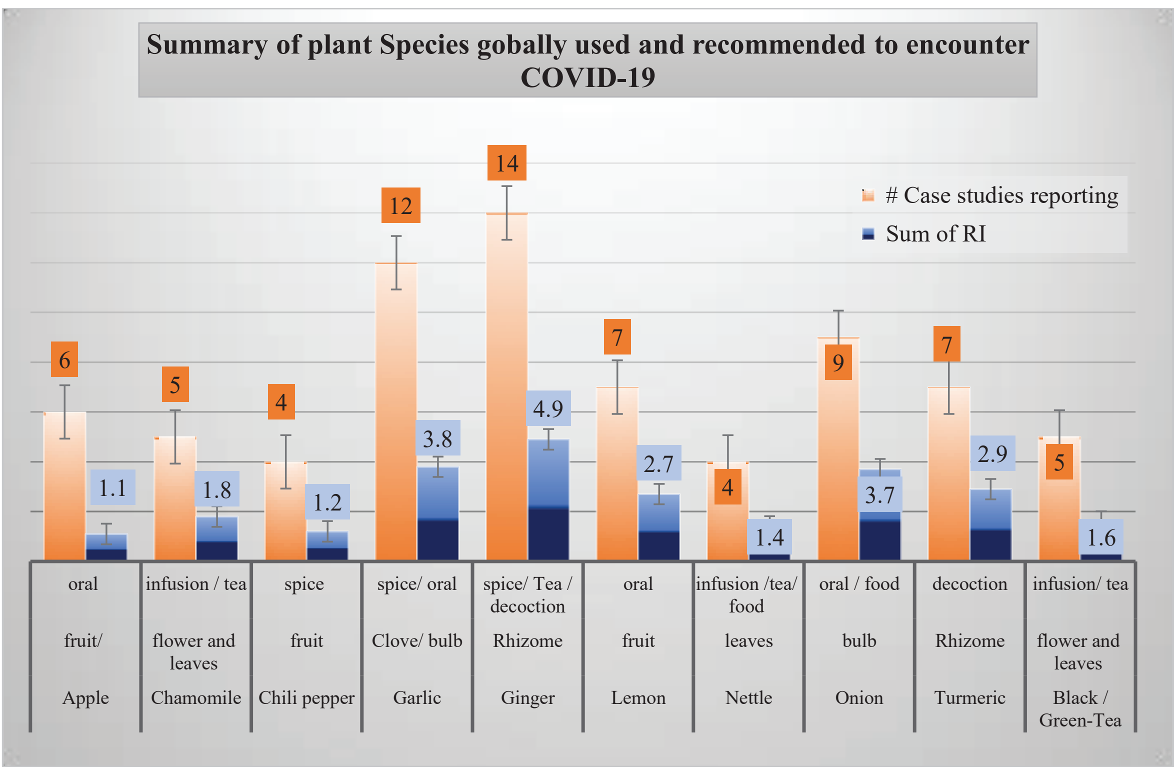

Fig. 4. Summary of globally utilized plant species and its mode of administration.

in organic than conventionally grown apples. The phenolic compound composition might be a possible reason for the recommendation against COVID-19 [39, 40]. Furthermore, genistein is found as the best phytoestrogen against a broad spectrum of viral diseases as reviewed by [41]. These medicinal plants could be acceptable as preventive measures against COVID-19 primarily by boosting the immune system and suppressing growth. Annual apple production in Pakistan was 620.0 thousand tonnes in 2016 from a land of 97,000 hectares. Apples are cultivated in Baluchistan, Khyber-

Pakhtunkhwa, Gilgit-Baltistan over 1000-meter elevation. Apple contributes significantly to livelihood and employment in the region.

4. CONCLUSION

The studies on internationally prioritized medicinal plants against the pandemic were evaluated. We have found that *Zingber officinale* Rosc, *Allium sativum* L, and *Allium cepa* L. were the potentially effective medicinal plants used in households to encounter the pandemic COVID-19. These species have anti-septic, anti-viral, and other therapeutic potentials that aid in the cure of diseases. These contain active secondary metabolites which can be the primary precursor in the drug discovery development against COVID-19. In case of any new variant of COVID 19 or a new pandemic, a country should think about the self-sufficiency in the nutraceutical products as lockdown may cause a severe problem in this connection. Therefore, we recommend further research on these plants to be evaluated which will lead to yield effective outcomes.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 67-76 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)657

Pakistan Academy of Sciences

Research Article

Public Awareness and Bio-management of COVID-19 by Four Medicinal Herbs in District Bhimber, Azad Kashmir, Pakistan

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Abstract: The current research work is focused on awareness and bio-management of COVID-19 through four selected natural medicinal herbs (MHs) in District Bhimber, Azad Jammu, and Kashmir (AJK), Pakistan. It was observed that the mortality rate is extremely high in people having weak immune systems, especially with pre-existing health problems. We have conducted questionnaire surveys protocol in different societies about COVID-19 assessment. After the field survey of the coronavirus, we used extracts of selected herbs against the patients of COVID-19 in a local hospital. In these preliminary experimental trials, we have tried to compile the effects of different MHs and their bioactive components that have the potential to combat the infection of COVID-19. The treatment of MHs can also be improved the immune system of the patients and future risk of viral attack can be minimized. The extracts of selected MHs (*Allium sativum* L, *Zingiber officinale* Roscoe, *Piper nigrum* L., and *Vitex negundo* L.) were given to already symptomatic patients with COVID-19 in the District Headquarter (DHQ) hospital Bhimber, AJK. The crude extracts of MHs showed 90 % positive results against COVID-19 patients in the preliminary experimental trials. The '*Allium sativum* and *Zingiber officinale*' were showed the best recovery rate with 95 % and 87 % respectively. Thus, it was proved that MPHs can help in the reduction of the mortality rate. Therefore, it is concluded that the MHs have also been considered as the best healing agents against this epidemic virus as well as boost-up the immune system of human beings. This article will help research laboratories and industries in the identification and scrutinization of potential medicinal herbs against COVID-19 and other viruses as well in the future.

Keywords: Medicinal herbs, Antiviral plants, Healing agents, Immunity enhancer, COVID-19.

1. INTRODUCTION

COVID-19 (Corona Virus Disease) was the first time originated in the province of Hubei, Wuhan City of our neighboring country (China) in the mid of December 2019. The first case of COVID-19 was observed from the person who visits the wet market of Wuhan City, China. Then the coronavirus was gradually spread in other Provinces of China and many other countries. Thus, the disease was declared as a global pandemic by World Health Organization (WHO) on 12th March 2020 [1].

It has rapidly spread throughout the world by traveling off the infecting people in different countries. The virus spread very quickly and become an epidemic in the whole world. The COVID-19 creating a lot of concern for people which leads to heigh-lightened levels of anxiety. So, the sudden exploration of the pandemics reached up to the levels of stress. This stressful situation is a common response to anxiety. Coronavirus disease gradually mutated and spread with a great infection rate and death ratio all over the world [2]. Identification of the Coronaviruses (CoV) was done first time in 1960. The disease CoV indicated mild influenza-like symptoms. These CoV diseases infected a wide range of vertebrates which includes snakes, birds, bats, camels, and many others [3]. However, it was observed that different virulent strains with emerging variables explored after few years causing deadly more epidemics [4].

According to WHO, a new virulent strain of virus known as SARS (Severe Acute Respiratory Syndrome) was identified in China. Then it was

Received: March 2021; Accepted: July 2021
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affected twenty-six (26) countries worldwide with 8098 cases in 2003. Similarly, another outbreak appeared with another strain of Coronavirus, known as MERS (Middle East Respiratory Syndrome) in 2012. The MERS-CoVi outbreak was explored from Saudi Arabia first time then suddenly spread into twenty-seven (27) countries and observed with 2494 cases worldwide [5, 6]. Another investigation indicated the establishment of the coronavirus in the year 1948 [7].

In the current study, we have tried to demonstrate the behavioral changes of humans due to the corona epidemic and their bad impacts on the public due to a lack of awareness about the COVID-19 virus. Here, "public awareness" elaborated much more information due to internet search at the public's level about the understanding of the COVID-19 [8]. Public awareness at the global level is also critically important because the empirical analysis is one of the major factors that support the diagnosis of the coronavirus. It is also considered more helpful in the prevention of the latest COVID-19 [9, 10].

Management of the coronavirus needs preliminary knowledge about the methods of disease transmission and their causal agents. This basic knowledge is important to produce very effective control measure strategies for viral diseases eradication/reduction [11]. Similarly, social awareness is also a key factor for the management of infectious diseases like a corona. Individual precautionary measures which include self-hygiene avoids from crowds, use of protective materials like sterile gloves and face masks are also reduce the chance of spreading the epidemic disease [12]. Therefore, these are facilities are helpful in the quick identification and treatment of new cases of the corona virus [13].

Some other preventive methods have been developed by various countries as self-isolation or self-quarantine at home, quarantine at country level, ban on public gatherings like schools, colleges, and universities, sealing borders, ban on transport, and also complete lockdown at city levels. With the help of the government, different control groups were formed in many countries that information about the individuals who suffer from fever, flu, and cough. Then government advice to those people for the COVID-19 test and also convey to them the possible preventive measures against the coronavirus. In this way, different organizations at the government level are trying to improve awareness of preventive measures like hand washes, the use of masks, and wearing gloves. Similar preventive measures were adopted by the Government of Pakistan against the COVID-19 to provide good safety tools for people. All services and control measures strategies were adopted when the first case of COVID-19 was confirmed in Karachi city of Sindh province in January 2020 and ensured the safe mode of life. It was observed that all cases of COVID-19 linked with the travel history of foreign passengers and their transmission almost imported from other countries. Therefore, The government of Pakistan tried best to provide mitigation strategies against the COVID-19 with their possible preventive measures [14].

The current scenario about the bio-management of COVID-19 is mainly focused on the preparation of vaccines in different countries and succeeded few countries but still, Pakistan did not able to prepare a vaccine for the pro-treatment of COVID-19. However, few other treatments are prescribed under different situations to minimize the severity rate of corona up to some level [15]. Alternatively, herbal treatments are considered very effective against COVID-19 with minimum side effects. Some antiviral agents were obtained from medicinal plant herbs (MPHs) in past.

Many herbalists explored various medicinal plants as anti-viral agents. It was observed that crude extracts of medicinal plants are used in their natural shape as first-generation drugs (FGD) or the active metabolites of plants are isolated for viral control as second-generation drugs [16]. However, more successfully, plant-based MPHs can address this issue of genetic variability of corona-virus in a better way. Herbs are also considered very competent for stunting the replication process of DNA viruses and RNA viruses within the specific/targeted host cells. The capability of herbal plants is positive to retard the survival of viruses in the host cells [16]. In contrast to synthetic drugs, some metabolites extracted from MPHs can reduce the replication process of the coronavirus without disturbing the metabolic mechanism of the respective host [17]. Coronavirus can be managing by the use of extracts from different MPHs in a better way. The following MPs have very effective constituents or metabolites against coronavirus such as *Glycyrrhiza radix*, *Dioscorea batatas, Psoralea corylifolia, Mollugo cerviana, Rheum officinale, Polygonum multiflorum, Trichosanthes cucumerina*, and *Salvia miltiorrhiza* [18, 19].

In another review, MPHs extracts of a plant named; *Tinospora cordifolia* has been used for patients infected by SARS-CoVi-2 strains [20]. Similarly, extracts from four different plants named; *Lindera aggregata, Artemisia annua, Lycoris radiata,* and *Pyrrosia lingua* have very dominant anti-viral potential in a host cell against the SARS-CoVi strain. It was also observed that chemicals like alkaloid and lycorine are present in *Lycoris radiata* which are maximum inhibitory potential against infection of SARS-CoV strain [20]. Phenolic compounds which are derived from plants also indicated anti-SARS-CoV activity in targeted host cells [21]. Another study has indicated that the extracts of *Houttuynia cordata* also considered very effective for the treatment of a virus named as SARS-CoV [22].

The primary objective of the survey by questionnaire method was to spread awareness about COVID-19 and prepared the mind of people for precautionary measures like handwashing with soap, use of gloves, sanitizers, and face masks, etc. These are few examples of herbal plants for the treatment of different types of viruses. Although, a lot of MHs are still unexplored against viruses. So, the current study mainly focused on MHs treatment against COVID-19 virus recovery. Precautionary measures are also compiled after a survey of a selected area by online questionnaire methods in this study before MHs treatment to the patients of COVID-19 in DHQ hospitals of district Bhimber, AJ&K.

2. MATERIALS AND METHODS

2.1 Questionnaire Method for Survey

The current research was focused on surveys of COVID-19 through an online structured questionnaire. The questionnaire method was used to collect information from different areas of District Bhimber, AJK during April and May 2020. The questionnaire protocol was developed on the educational material published by the World health organization (WHO). The questionnaire was divided into the following parts as; i) Demographic data of all participants, ii) General awareness about the COVID-19 disease, iii) Knowledge of COVID-19 symptoms, iv) To measure public knowledge about the COVID-19 disease transmission, v) Documented the preventive measures, vi) Gathered information about the treatment and practices of the COVID-19 patients, vii) Collected knowledge about the herbal medicinal plants (HMPs) treatment and compared with previous literature. Responses of the questionnaire were reported in percentage (Table 1) as well as "Yes" or "No" (Table 2). The participant was given a percentage score ranging from 1 to 100%. The participants were classified as having good knowledge about the disease if he/she scored >80 % which was considered a more satisfying percentage in the most previous literature survey. The socio-demographic details that included gender, age groups, occupational level, and education sectors of different regions have been selected for the COVID-19 survey [2, 23]. In this way, we were able to collect data from across various areas of District Bhimber, AJ&K.

2.2 Management through Herbal Medicinal Plants Treatments

Four medicinal plant herbs (MPHs) were selected for the treatment of COVID-19 because these plants are already used against different viral diseases in literature [32-35]. Therefore, these MPHs extracts were used against COVID-19 and received promising results. The extracts of fruits and seeds of *Piper nigrum* were prepared with 5% and 8% concentrations for the treatment of COVID-19 [24]. Piperamides metabolites present in the seeds of the piper plant which is responsible for anti-viral activity [25]. Leaf extracts of *Vitex negundo* were also prepared at the rate of the same concentration as mentioned above. Before extracts preparation, whole plants were dried and prepared powdered. the powder was soaked in distilled water for 2 to 3 hours. The prepared extracts in distilled water were taken orally. Chinese took powder of *V. negundo* with daily tea [26]. *Allium sativum* extract was also prepared from underground stem/bulb at 5% and 8% concentration in sterilized water. The fourth plant extract was prepared from their rhizomes.

Table 1. Survey of COVID-19 awareness by different type of participants.

Level	Characteristics	Query	Percentage (%)	Awareness Scoring (Mean ± SD)
Gender	Men	301	34.09	24.31 ± 1.06
	Women	582	65.91	27.57 ± 1.79
Ages (years)	16-26	530	13.52	17.34 ± 0.86
	27-37	1250	31.89	21.48 ± 1.01
	38-48	1040	26.53	20.76 ± 2.05
	49-59	750	19.13	16.82 ± 0.95
	≥ 60	350	8.93	11.03 ± 1.02
Occupational groups	Teachers of public institutions	550	12.47	16.87 ± 1.00
	Teachers of Private institutions	300	6.80	10.75 ± 1.52
	Students (Male)	1180	26.76	18.56 ± 2.00
	Students (Female)	930	21.09	19.98 ± 1. 85
	Retired persons	220	4.99	8.43 ± 0.81
	Health workers	160	3.63	6.17 ± 0.54
	Local Govt. workers	670	15.19	11.58 ± 0.94
	Industry workers	400	9.07	9.64 ± 0.69
Educational Level	Primary Schools	350	7.43	8.69 ± 0.37
	Middle Schools	570	12.10	10.11 ± 0.96
	High Schools	760	16.13	12.45 ± 1.00
	Intermediate Colleges	1230	26.11	21.48 ± 1.87
	Degree Colleges	1050	22.29	19.25 ± 1.54
	Postgraduate Colleges	750	15.92	11.85 ± 1.00

Zingiber officinale extract was used in concentrated form. The extract of *Zingiber officinale* blocked viral attachment and it was reported in the literature as a good inhibitor of human respiratory syncytial virus (HRSV) as followed Mao *et al*. [27] with some modifications.

These all prepared extracts were used in preliminary experimental trials in District Headquarter Hospital (DHQ), Bhimber, AJ&K for the treatment of COVID-19 patients in the quarantine ward. The results were measured and documented after one week on basis of symptoms.

3. RESULTS AND DISCUSSION

COVID-19 belongs to a large coronavirus family and is well known by name of virulent Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) virus. The current research is focused on a survey of COVID-19 by demographic analysis and management through treatments by local herbs in District Bhimber, AJ & K, Pakistan. Scientists are mainly focusing on herbal treatments for the cure of the epidemic viral disease because these bio-treatments have no side effects. Sometimes, medicinal plants do not effective against the specific target disease but their use did not show

Table 2. A general survey of the coronavirus disease (COVID-19) and the analysis rating.

Sr. No.	Questionnaire for Public Awareness	Answer (%)
1	Whether you observe COVID-19 in people around you or not?	Yes :- 20.11 % No :- 79. 89 %
2	Are you knowing well about basic knowledge of the COVID-19 virus?	Yes :- 80.45% No :- 19.55 %
3	Is COVID-19 responsible for respiratory disorder?	Yes :- 90.34 % No :- 9.66 %
4	Hand-washing with soap has the same effect as without soap from preventing the spread of the COVID-19 virus?	Yes :- 14.0 % No :- 86.0 %
5	Is the COVID-19 virus cause of death for patients with a chronic disease?	Yes :- 75.08 % No :- 24.92 %
6	The incubation period of the COVID-19 virus is 14 days?	Yes :- 98. 55 % No :- 01.45 %
7	Do patients feel respiratory symptoms before the COVID-19 attack?	Yes :- 18.67 % No :- 81.33 %
8	Is the COVID-19 virus an airborne viral infection?	Yes :- 97. 45 % No :- 2.55 %
9	We have enough hospitals, doctors and ventilation instruments to face the epidemic COVID-19 virus disease?	Yes :- 16.38 % No :- 83.62 %
10	We are spreading information about the COVID-19 virus between our family and friends or not?	Yes :- 72.41 % No :- 23.59 %
11	What is the recovery ratio of the COVID-19 by herbal extracts treatment?	Yes :- 65.82 % No :- 34.18 %
12	The high temperature in summer may kill the COVID-19 virus **or** not**?**	Yes :- 41.78 % No :- 58.12 %
13	The Ministry of Health considered the COVID-19 virus as an infectious disease or not?	Yes :- 98.17 % No :- 1.83 %
14	The COVID-19 may be a repeat cycle in a more severe form in the future?	Yes :- 93.69 % No :- 6.31 %

any negative impact on the body. Therefore, with the emerging methodology of vaccines and other treatments, herbal treatment is recommended as one of them. The herbal mixture was recommended by the Chinese National Health Commission (CNHC) for the management of COVID-19 [4]. It means that extracts of medicinal plants are observed as effective treatments of the virus.

The levels of demographic data analysis composed of gender interviews, different age level discussions, occupational level, and education sectors survey as mentioned in Table 1. It was observed that a total of 883 male and female participants were involved in the survey. The demographic data of the participants indicated a maximum percentage (65.91 %) of women as compared to men (34.09 %). The family group of ages 27-37 years with 31.89 % of the informants know well about the clinical symptoms of COVID-19. The awareness about COVID-19 was observed maximum (26.76%) by male students in educational groups. Awareness creation about COVID-19 was observed higher (26.11%) at the intermediate college level (Table 1). Similar findings were explored by Elgendy *et al.* [28]. Awareness about the virus is the best way to reduce their infection or patients.

A 14 question survey was conducted about the exploration of COVID-19 awareness. It was observed that maximum peoples (79.89%) were aware of the pandemic of the virus. Most of the people knowing the COVID-19 (80.45%). During the survey, it was also depicted that 90.34% of people considered COVID-19 positive. Maximum peoples were also aware well about precautionary measures like washing hands with soap (86.0%). Some other questions were also explored and documented their answers as shown in Table 2. These findings were strongly supported by Alahdal *et al.* [29].

Medicinal Herbs (MHs) crude extracts contained several metabolites which have the potential for an effective anti-viral drug. Many different anti-viral metabolites of plants including peptides, terpenoids, lignin, flavonoids, polysaccharides, polyacetylenes, and alkaloids were very effective against different targeted viruses [30, 31]. In the current research, four herbal plants named; *Allium sativum*, *Vitex negundo*, *Piper nigrum*, and *Zingiber officinale* were selected for antiviral activity. The responses of each medicinal plant documented in Table 3 from previous literature with their references as justification [32-35]. After their confirmation from literature, these plant extracts were used against the epidemic virus in hospital trials for analyzing the bio-efficacy of selected MHs directly.

The potential of the four MHs were measured and compiled results about COVID-19 virus recovery rate (%) after their treatments. The first treatment is given with 5-gram concentration treatments for three days and it was observed maximum recovery rate (90 %) with *Allium sativum* treatment. *Zingiber officinale* extracts also indicated better results with an 84 % recovery rate. In the second trial, we observed the recovery rate of COVID-19 patients at a concentration of 8gram extract treatment. The two herbs '*Allium sativum* and *Zingiber officinale* were also indicated the best recovery rate of 95 % and 87 % respectively. These findings were measured by using crude extracts of the selected herbs. Hence, these herbs may have chemicals/metabolites with the potential for COVID-19 virus recovery. These findings were supported by different microbiologists [32, 33]. These herbal treatments have no side effects. Therefore, most people were preferred herbal

Table 3. Antiviral activity of herbal medicinal plants

Herbal Plants	**Antiviral responses**	**References**
Allium sativum	Antiviral, Proteolytic and hemagglutinating activity	Balachandar *et al.* [32]
Zingiber officinale	Antiviral activity, It combats drug resistance in antivirals against CHIKV.	Kaushik *et al.* [33]
Piper nigrum	Inhibitory effect against COVID-19.	Narkhede *et al.* [34]
Vitex negundo	It inhibits the Chikungunya virus and active against asthma, cough, bronchitis, headache, fever, and influenza.	Khanal *et al.* [35]

Table 4. Responses of selected medicinal herbal plants against COVID-19 in hospital trials

Plants Used	**Recovery rate (%) of COVID-19 in hospital trials**			
	Dosage (5 g)		Dosage (8 g)	
	Treated Patient	Untreated Patient	Treated Patient	Untreated Patient
Allium sativum	90 %	10 %	95 %	5 %
Zingiber officinale	84 %	16 %	87 %	13 %
Piper nigrum	70 %	30 %	75 %	25 %
Vitex negundo	65 %	35 %	68 %	32 %

treatments against the COVID-19 virus as well as other viral and fungal diseases.

4. CONCLUSION

This study has been focused on the awareness creation among peoples and bio-management of COVID-19 through four selected MHs. This study was spread the general public awareness about the protective measures against viral disease. It was concluded that MHs showed promising results against the virus-infected persons in preliminary trials and considered good alternatives to prevent COVID-19 disease effectively in the local hospital of district Bhimber. Hence, the overall 80 % potential response of four medicinal herbs was calculated against the outbreak of the epidemic disease of COVID-19. As the two MHs '*Allium sativum* and *Zingiber officinale*' were declared best against COVID-19 with a recovery rate of 95 % and 87 % respectively. Therefore, we should focus more on MHs for the treatment of viral diseases like COVID-19. Researchers should focus on specific compounds isolated from these MHs for the management of target disease effectively in the future.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 77-78 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)WebI

Pakistan Academy of Sciences

Proceedings of the AASSA-PAS Webinar I on Sub-Theme: SDGs and Pandemics

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Pakistan Academy of Sciences (PAS) and the Association of Academies and Societies of Sciences in Asia (AASSA) with the support of InterAcademy Partnership (IAP) organized an AASSA-PAS Webinar I on SDGs and Pandemics" on April 27, 2021, in the Pakistan Academy of Sciences. H.E. Senator Shibli Faraz, Federal Minister for Science and Technology inaugurated the Webinar.

Prof. Khalid Mahmood Khan, President Pakistan Academy of Sciences presented the welcome address. He emphasized the importance of media and sharing of data to counter the emerging pandemic potential. The Minister of Science & Technology, Senator H.E. Shibli Faraz appreciated the efforts of PAS, AASSA, and IAP for timely taking up the important issue of Pandemics. He inaugurated the webinar and mentioned that among the most pressing issues in preparing for the global response to a pandemic situation are the design, development, manufacture, and dissemination of vaccines. The response to this pandemic has shown that when leading vaccine manufacturers are fully engaged in a global response, it might be possible for them to manufacture substantial doses of vaccine on timelines faster than envisioned previously. Prof. Yoo Hang Kim (President AASSA) reiterated his support to the academies in Asia for holding such events and containing coronavirus in the region. There is a flood of information on COVID-19. But misinformation is, unfortunately, a big part of the problem we face in confronting the pandemic.

Under the sub-theme "SDGs and Pandemics", experts from four countries (Turkey, UAE, Nepal, and Pakistan) shared their views. The speakers covered the themes of the role of collaborative efforts of Science academies in the preparedness of pandemics; the impact of Covid-19 on women and publishers and their role in facilitating the publishing and engaging scholarly community. Some other focus areas were:

- Dealing of Virus Infected Biological Samples using Modern Mass Spectrometry Tools: Hepatitis C Virus as an Example
- Tracking the pandemic in Sindh: From training and Capacity Building for COVID-19 testing to Exploring the Emerging Variants
- COVID-19 pandemic and other co-epidemics: a challenge for the overburdened healthcare system in developing countries
- COVID-19 pandemic as a test case in the anthropocene epoch; the interplay of environment, ethics, and psychology on the global stage
- Alternative Therapies for Pandemic Diseases using Herbal Drinks
- Artificial Intelligence and Predictive Survival Analysis for Covid-19
- Development of an Assessment Method for Investigating the Impact of Climate of Lahore on Confirmed Cases of COVID-19

The main recommendation from the webinar was on "Building the Global Vaccine Manufacturing Capacity" to respond to Pandemics" and equity issues of Low and Middle-Income Countries. A nationalistic rather than global approach to vaccine delivery is not only morally wrong but will also delay any return to a level of "normality" (including relaxed border controls) because no country can be safe until all countries are safe.

Way Forward

The organizer of the event, Prof. Zabta Khan Shinwari spoke about the way forward to combat COVID-19. He remarked that what emerges next will partly depend on the ongoing evolution of SARS-CoV-2, on the behavior of citizens, on the government's decisions about how to respond to the pandemic, on progress in vaccine development (its distribution, WTO regime, and IPR issue) and treatments, and also in a broader range of disciplines in the sciences and humanities that focus both on bringing this pandemic to an end and learning how to reduce the impacts of future zoonosis (focusing on One Health), and on the extent to which the international community can stand together in its efforts to control COVID-19. Many factors will determine the overall outcome of the pandemic.

Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 79-80 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)WebII

Pakistan Academy of Sciences

Proceedings of the AASSA-PAS Webinar II on Sub-Theme: Pandemics Preparedness, One Health: Lessons Learnt

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Pakistan Academy of Sciences (PAS) and the Association of Academies and Societies of Sciences in Asia (AASSA) with the support of InterAcademy Partnership (IAP) organized an AASSA-PAS Webinar II on **Pandemics Preparedness, One Health: Lessons Learnt"** on May 4, 2021, in the Pakistan Academy of Sciences. Speakers from Pakistan, Bangladesh, China, Indonesia, Japan, Turkey, and Sri Lanka delivered talks. **Dr. Fazal e Hadi, Chairman Islamabad Healthcare Regulatory Authority** inaugurated the Webinar. **Dr. Fazal e Hadi** noted that the Islamabad Healthcare Regulatory Authority (IHRA) is an autonomous health regulatory body enacted under the Islamabad Health Regulation Act, 2018, and discussed the efforts of the IHRA for educating and making people aware to follow standard operating procedures (SOPs) and standardize their living to counter this pandemic. He stressed on the need to provide evidence for awareness. He recommended that strengthening of leadership and provision of vaccines to everybody is the only way forward to combat the pandemic.

Prof. Khalid Mahmood Khan, President of the Pakistan Academy of Sciences welcomed all the speakers, guests, and organizers of the webinar. He appreciated the efforts of PAS and AASSA for arranging these webinars in this difficult time of the COVID-19 pandemic. **Prof. Yoo Hang Kim, President AASSA,** appreciated the collective efforts of various stakeholders in organizing the webinar for the awareness and strengthening of SOPs across the world. He recommended inviting more people in the future to speak about this epidemic. He emphasized on making people aware of this pandemic. **Prof. Zabta Khan Shinwari,** Chief Organizer of the Webinar, thanked all the speakers of the Inaugural Session and the Chief Guest for being part of the Webinar Series. He explained scientific reasons as to why to observe social distancing. **Coronavirus drifts through the air in microscopic droplets: the science of the infectious aerosols.** Wearing masks and practicing social distancing can, in large part, prevent people from spreading or inhaling aerosols.

Prof. Muhammad Ali (Vice Chancellor, Quaid-i-Azam University, Islamabad, Pakistan) and Prof. Zabta Khan Shinwari (Chief Organizer of the Webinar) served as the moderators of this webinar. Different countries shared their experiences on covid-19. **Prof. Nariyoshi Shinomi,** from Japan, highlighted the current situation of COVID-19 in Japan, while **Prof. Zabta Shinwari** sensitized the participants about biosecurity & neglected aspects of pandemics in Pakistan. The keynote address by **Prof. Jinghua Cao** (Executive Director, Alliance of International Science Organizations, ANSO) discussed the efforts of ANSO members to counter the COVID-19 epidemic. He emphasized the role of ANSO to bring people together and promote solidarity and unity within ANSO to oppose the spread of this epidemic collectively. **Prof. Dr. Mehmet Bulut (Member, TUBA)** emphasized the importance and role of social finance in achieving SDGs objectives. He expressed the need for sustainable development that incorporates meeting the needs of the present and future. Other issues discussed included:

- Vaccinomics and probable prevention strategy against avenging zoonotics
- Nanotechnologies for COVID-19 theranostics
- Development of biodegradable surgical facemask made of marine algae to confront COVID-19 pandemic challenges.

- Understanding COVID-19 pandemic concerning anti-scientific and pseudoscientific world views
- The effective role of the scientists to counter the infodemic
- Practices of complementary & alternative medicines in COVID-19 pandemic

While giving concluding remarks Prof. Zabta Shinwari, emphasized on collective working to share data and lead the research on the pandemic to reduce the overall impact of this epidemic. All the stakeholders and researchers should work together to collaborate and find out how epidemics can be controlled and the economy can be improved. He gave recommendations on regulating media, proper law enforcement, and handling misinformation to support each other against this pandemic.

The consensus emerged on "A One Health Approach to Preventing the Next Pandemic". Instead of waiting for the next deadly microbe to spill over into humans, public health experts and policy-makers must confront the drivers of zoonotic diseases. Some countries have a long tradition of people eating wild animals and using them in traditional medicines—practices that likely increase the transmission risks of microbes from animals to humans, causing what are called zoonotic diseases. The beginning of this pandemic is reminiscent of another disease caused by a coronavirus, severe acute respiratory syndrome (SARS), which also originated in a live animal market in China. A chef who regularly cooked with exotic animals was one of the earliest persons to be diagnosed with SARS. A coronavirus is also responsible for causing Middle East respiratory syndrome (MERS). MERS viral genomic studies suggest that the virus is endemic to camels in Saudi Arabia. With a mortality rate estimated at nearly 35%, MERS is much deadlier than its coronavirus cousins but more difficult to contract. For MERS, the initial camel-to-human transmission appears to have occurred via nasal secretions from four sick camels.

Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 81-83 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)WebIII

Pakistan Academy of Sciences

Proceedings of the AASSA-PAS Webinar III/Hybrid Workshop on Sub-Themes: How to Counter Infodemics: Role of Scientists and Role of Complementary Medicine in Pandemics & Diagnosis, Critical Care; Vaccines and Herd Immunity"

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The Pakistan Academy of Sciences (PAS) and the Association of Academies and Societies of Sciences in Asia (AASSA) with the support of InterAcademy Partnership (IAP) organized an AASSA-PAS Webinar III/ Hybrid Workshop on 25 May 2021, in the Pakistan Academy of Sciences. The webinar/Hybrid Workshop covered specific themes i.e. **"How to Counter Infodemics: Role of Scientists & Role of Complementary Medicine in Pandemics"** and **"Diagnosis, Critical Care; Vaccines and Herd Immunity"**. In total, 13 lectures were delivered by leading experts, of which five were delivered by international speakers from Iran, Korea, Russia, USA, and Vietnam and eight by Pakistani speakers. **H.E. Zartaj Gul, Minister of State for Climate Change, Government of Pakistan (GoP)** was the Chief Guest of the Webinar.

Prof. Khalid Mahmood Khan, President-PAS, welcomed the guests and participants and appreciated the endeavours of PAS, AASSA, IAP, the organizers, participants and webinar attendees to cooperatively organize these webinar series and discussed the role of these webinars for improving the situation in this epidemic. **Prof. Yoo Hang Kim, President-AASSA,** appreciated the efforts of AASSA, PAS, Prof. Zabta Khan Shinwari, the organizers, participants (both at the venue and online), and all the people who contributed to the webinar. He gave various recommendations for COVID-19 that included global coordination, global organization, and adoption of all the safety and biosecurity measures suggested by the World Health Organization (WHO).

In her inaugural address, **H.E. Zartaj Gul, Minister of State for Climate Change, Government of Pakistan** extended her thanks to AASSA, PAS & IAP, and all the organizers for arranging the webinar, and to guests of honour, faculty, and participants for attending the sessions. Given the adverse impacts of COVID-19, she emphasized arranging more webinars on the topic in the future. She further added that science and policy-making are two very distinct cultures. Hence, someone has to bridge this gap and the Pakistan Academy of Sciences is already playing this pivotal role effectively. Hence, there is increasing recognition of the importance of boundary roles and structures in linking these cultures. The question is how to achieve the targets of SDGs?; will a new set of goals help the world shift from a dangerous business-as-usual path to one of truly sustainable development? With the growing fear of pandemic and epidemic infectious diseases, scientists are using biotechnology to develop new diagnostic tools for rapid and sensitive detection of pathogens. In conclusion, on behalf of the Ministry of Climate Change, she assured full support and all-out help to see Pakistani's collaboration in all aspects to control the current crises due to COVID-19.

Prof. Tasawar Hayat, Secretary General PAS gave a brief account of the Pakistan Academy of Sciences (PAS) in terms of its aims and objectives and key success stories. **Prof. Zabta Khan Shinwari, Chief Organizer of the Webinar,** appreciated the efforts of ASSAA and young scientists at PAS in arranging this webinar. He announced a prize that was constituted by the president of AASSA for the best poster and appreciated the number of posters being presented. He also informed that the webinars in the series had attracted an average of nearly 500 registered participants from 15 different countries. He also appreciated the efforts of IAP for sponsoring such events and producing reports on pandemics that are guidelines for preparedness (https://www.interacademies.org/publication/interdisciplinary-research-epidemic-preparedness-and-response). **Prof. Dr. Shahid Mahmood Baig** (Chairman, PSF) and **Maj. Gen. Aamer Ikram** (Executive Director, NIH) were the moderators of the Technical Sessions. Lessons learnt from Canada, Korea, Pakistan, Russia, USA, and Vietnam and were shared.

Other issues discussed included:

- COVID-19 and priorities for research & development: findings from a PAS Round Table
- COVID-19 Vaccines; Pakistan's perspective
- Utilitarianism Ethics and COVID-19: resource allocation and priority-setting
- Plant Biotechnology; an important avenue for medicine against COVID-19 and future pandemics
- Understanding COVID-19 pandemic with reference to anti-scientific and pseudoscientific world views
- The Effective role of the Scientists to counter the Infodemics
- Practices of Complementary Medicine in the COVID-19 Pandemic
- Efficacy of Different Treatment Regimens against the Symptoms of COVID-19: A cross-sectional study
- COVID-19 Response in the Republic of Korea
- Fight against Viruses (COVID-19): Peace among nations
- Digital Hygiene against Infodemics
- Building the Global Vaccine Manufacturing Capacity needed to Respond to Pandemics
- COVID-19 Pandemic Control: Lesson from Vietnam

The concluding session of the Workshop was chaired by **H.E. Masood Khan, President AJ&K**. In his address, **H.E. Masood Khan** emphasized on parameters such as convening power, evidence of preparedness, state-based ownership, technological evidence, and codes of conduct to ensure the security of people in this epidemic. He encouraged promoting the role of scientists to counter the epidemic. He congratulated Prof. Dr. Zabta Khan Shinwari for the timely organization of this international webinar. He also recognized the concrete recommendations made during the webinar series by the expert faculty and participants from Pakistan and around the world. He added that Covid-19 has been the most potent and destabilizing threat to our health systems this century, causing huge losses of human lives and a deficit of trillions of dollars in national economies. The latest count shows 167 million cases worldwide and 3.46 million deaths. In hindsight, it is clear that the entire world, including the countries with the most advanced health systems, was not ready for Covid-19. We had forgotten about outbreaks of SARS, MERS, and Ebola because they were confined to certain regions and were contained. Lessons learnt from the Russian Flu in the 1890s and the Spanish Flu in 1918 were buried in the pages of history. So this novel virus struck ominously and without prior warning, but it has taught us a hard lesson that this is not the last global outbreak and that we have to be prepared to deal with future emergencies which can ruthlessly destroy lives and tear apart global and national economic and social fabrics. The pandemic has demonstrated once again our interdependence and mutual vulnerability necessitating a coherent and cohesive approach at the national, regional, and international levels. **A threat to even one individual is a threat to us all**. What is puzzling is that nations with fewer resources were able to fight better than the ones equipped with the most advanced biomedical facilities. Finally, scientists should be given a pivotal role in fighting pandemics.

Maj. Gen. Aamer Ikram explained the present situation of Covid-19 in Pakistan and the efforts of the Pakistani government to contain it and reduce the damage. Prof. Dr. Zabta Khan Shinwari discussed social safety, economy, the One Health concept, CAM therapies, vaccinomics and vaccinations, the SDGs, social financing, nanotechnology, ethics, biodegradation, the use of surgical masks, and how the PAS had successfully achieved the goal of discussing all the major objectives of the webinar series in detail. He mentioned that scientists are collaborating to make vaccines to combat COVID-19 and discussed the collaborations of AASSA-PAS with UNESCO, WHO, and all the social issues that can overall increment the impact of COVID-19.

Finally, as the conclusion of the webinar series, Prof. Tasawar Hayat, PAS Secretary General, announced the gold, silver, and bronze medals for the best posters by young graduates in the Poster Competition. The prize money was donated by the AASSA President. A partial contribution was also made by the organizers of the workshop.

Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 85-87 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(58-sp1)WebIV

Pakistan Academy of Sciences

Proceedings of the AASSA-PAS Webinar IV/Hybrid Workshop on COVID-19 and Higher Education: Addressing Food Insecurity through Policy Support and Research

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The Pakistan Academy of Sciences (PAS) and the Association of Academies and Societies of Sciences in Asia (AASSA), with the support of the InterAcademy Partnership (IAP), organized an AASSA-PAS Webinar VI/Hybrid Workshop on **COVID-19 and Higher Education: Addressing Food Insecurity through Policy Support and Research:** 24 June 2021, in the Pakistan Academy of Sciences. In total, 13 lectures were delivered by leading experts, of which four were delivered by international speakers from Australia, Canada, China, and the UK, and eight lectures by Pakistani speakers. **H.E. Syed Fakhar Imam,** Federal Minister, National Food Security, and Research, inaugurated the webinar.

Prof. Khalid M. Khan, President Pakistan Academy of Sciences (PAS), welcomed the guests, speakers, organizers, and webinar participants to the fourth workshop of this series on Pandemic Preparedness, Science, and Countermeasures. He added that in the previous three webinars, PAS had successfully covered important aspects including gender, poverty, food, and marginalized income, and other aspects to create awareness among the public. He thanked **Federal Minister for National Food Security & Research, Syed Fakhar Imam** for managing time to grace the occasion with his online participation and to inaugurate the event.

Prof. Zabta Khan Shinwari, Treasurer-AASSA, and Fellow PAS appreciated the efforts of AASSA-PAS, organizers, attendees, and speakers to collectively participate in this webinar and for their communication about the topics for webinar-IV. He recognized the participation of all in this webinar and emphasized arranging other similar webinars in the future with the collaboration of AASSA and IAP. He thanked all the eminent personalities and other participants from various ministries of the Government of Pakistan for their physical presence to participate in this webinar. **Prof. Yoo Hang Kim, President of AASSA**, acknowledged the efforts of AASSA for organizing this webinar. He expressed his gratitude to **President PAS and Treasurer AASSA** for their cooperation to make this webinar a success.

In his keynote address, **Prof. Viktor Bogatov, Academician of Russian Academy of Sciences and Chief Academic Secretary of Far Eastern Branch of Russian Academy of Sciences,** discussed the imbalance in COVID-19 cases across the world and emphasized the role of science and technology in tackling the pandemic.

H.E. Syed Fakhar Imam, Federal Minister, National Food Security, and Research, Government of Pakistan, inaugurated the webinar and highlighted the impact of the pandemic on people. He conceded the role of institutions and the armed forces to maintain, organize and collect statistics about the pandemic from all over the world. He expressed his desire to improve exports from Pakistan's food and agriculture sector. He added that some 8.4 million tons of rice, 8.4 million tons of maize, and 1 million tons of sugarcane are produced, contributing to GDP growth in Pakistan, and said emphasis should also be on food and livestock production.

Prof. Dr. Tasawar Hayat, Secretary General, PAS thanked Prof. Dr. Zabta Khan Shinwari (Chief organizer of the Webinar Series) for his contributions to the organization of the webinar.

Prof. Dr. Iqrar A. Khan (VC-UAF), Prof. Dr. Kausar A. Malik (Fellow, PAS), and Prof. Dr. Zia Ul-Qayyum (VC, AIOU) were the moderators of the Technical Sessions. Speakers delivered lectures on different issues and topics:

- Food Security Challenge and Options
- Enhance Food and Nutritional Security
- Plant Genetic Resources Management under Pandemic Constraint Context
- CropWatch for Ensuring Food Security under COVID-19
- Soybean as an Intercrop
- Precision for Efficient Agriculture
- Biotech Role in Ensuring Food Security
- COVID-19: Higher Education challenges and responses
- No student left behind in remote areas like Waziristan with less or no Internet connectivity in pandemic Covid19
- Way forward for Higher Education in Pandemics
- COVID-19 Reshape the World: Can it be a positive catalyst for ecological sustainability and Sustainable Development Goals: A case study from Swat
- Post-Covid Higher Education: Responses, Recovery, and Resilience.

In the concluding session, Prof. Yoo Hang Kim, President-AASSA, appreciated the efforts of the AASSA-PAS webinar organizers, participants, and speakers to make this event a success. **Mrs. Farah Hamid Khan, Federal Secretary, Education, and Professional Training,** Chief Guest of the **Concluding Session**, expressed her views about misinformation in the epidemic. She suggested exploring new ways of learning to standardize the provision of education to the people. She was happy to notice that the webinar focused on educators and researchers in different parts of the world to find out how COVID-19 has affected them and how they are coping with the changes. She expressed that, through this webinar, we will also be able to highlight lessons learned and potential positive outcomes of the global lockdown for higher education. She further added that these workshops are forums at which we share our experiences, successes, and failures as well, and learn from each other for the benefit of humankind. The valuable lessons we learned at this workshop must not remain only among ourselves. These must be propagated as widely as possible. I also urge this workshop, through the collective intelligence of the participants, to formulate good recommendations to all stakeholders, including policymakers, scientists, and the general public. In conclusion, Mrs. Farah Hamid Khan thanked all the delegates (who joined virtually) and assured the audience that from the Government's side, ministry authorities shall always be there to assist PAS and other institutions in endeavours to combat the pandemic.

Prof. Zabta Khan Shinwari gave recommendations for countering this epidemic. While appreciating the efforts of AASSA and IAP for supporting such events, he said recommendations of the current webinars will add to the IAP communique: https://www.interacademies.org/publication/iap-communique-covid-19 about covid-19. He suggested the realization of the needs of students' learning and encouraged universities to revive their policies for providing education in this epidemic as students face internet connectivity issues in far-off places. He proposed collaboration and countering of misinformation to fight against this epidemic and recommended for provision of the following opportunities to students and others:

- Awareness to students about various communication platforms for learning.
- Peer mentors and student leaders are a need of the hour to cultivate a sense of learning.

- Provision of opportunities to people to share their experiences and engaging them with necessary tools to make learning more efficient.
- Encouraging collaboration and understanding all the problems of students would help in standardizing the culture of learning at the national level.
- Provision of vaccines to all people within our community.

Towards the end of the concluding session, Medals/Cash Prizes were distributed among the winners of a poster competition.

Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 58(S): 89-91 (2021)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X

Pakistan Academy of Sciences

Recommendations

AASSA-PAS Webinar Series / Hybrid Workshops 2021 Recommendations

The AASSA-PAS Webinar Series 2021 on **"Pandemic Preparedness: Science and Countermeasures"** was organized jointly by the Pakistan Academy of Sciences (PAS) and the Association of Academies and Societies of Sciences in Asia (AASSA) with the support of the InterAcademy Partnership (IAP). The series consisted of four webinars, each covering a specific theme of the current pandemic scenario/situation and scheduled on 27 April 2021, 4 May 2021, 25 May 2021 and 24 June 2021. The webinars provided an opportunity to share lessons learnt among the participating countries and scientists about COVID-19 and its relation to the UN Sustainable Development Goals (SDGs); strategies for the current issues and challenges while facing pandemics; and the increasing awareness about the preparedness of the future pandemics. Use of emerging technologies like Artificial Intelligence applications in tracking health behaviours during disease epidemics and encouraging the use of ICT technologies and social media for tackling the spread of misinformation regarding different aspects of the pandemic were discussed as wel las issues of biosafety, biosecurity and ethics.

In total, 48 lectures were delivered in the AASSA-PAS Webinar series 2021 by leading experts. Of these, 18 were presented by international speakers and 30 by speakers from Pakistan. The resource persons in the webinars were leading foreign experts from different countries i.e., Australia, Bangladesh, China, Indonesia, Iran, Japan, Korea, Kyrgyzstan, Malaysia, Nepal, Pakistan, Russia, Turkey, United Arab Emirates (UAE), USA and Vietnam. More than 2,000 national and international participants registered to participate in the AASSA-PAS Webinar series 2021 (including 748 participants in Webinar I, 760 participants in Webinar II, 428 participants in Webinar III, and more than 300 in Webinar IV). At the conclusion of the AASSA-PAS Webinar Series, participants worked together to develop a list of recommendations that will further help in creating a better and more robust pandemic response. These recommendations are:

1. A global vaccination drive for COVID-19 should be considered as the top priority. However, vaccine safety should be ensured. Advanced countries with the capacity to manufacture vaccines should assist in building the capacity for vaccine manufacturing in developing countries.

2. COVID-19 vaccinations should be made available to everyone. However, governments should ethically ensure their availability and distribution. International organizations like the World Health Organization (WHO) should take the lead in providing COVID vaccines across underdeveloped and developing regions, especially the Least Developed Countries (LDCs) and Low- and Middle-Income Countries (LMICs).

3. COVID-19 passports could play an important role in reopening societies and restoring the civil liberties that were reduced to mitigate the spread of the virus. But at the same time, they bring important ethical concerns. COVID-19 passports can bring unjust forms of exclusion that should be avoided. Given the global inequality in access to vaccines, the introduction of COVID-19 passports could lead to a deepening of global divides. Unjust travel limitations for those who did not have access to vaccines should be avoided. The unequal treatment of people based on having and not having a COVID-19 passport can cause a stigma and a social dichotomy (Adopted from the UNESCO recommendation on COVID-19 passports).

4. Despite impressive scientific achievements, barriers such as the vaccine cold chain and multiple forms of intellectual property (IP) protection like TRIP-WTO stand in the way of equitable access

and fair allocationof vaccines and other medical technologies. These need to be relaxed especially for pandemics, which will encourage LMICs to develop their own vaccines, etc.

5. While a global vaccination drive is extremely important to build immunity for COVID-19, these vaccinations must not mask other mass vaccination programmes, for example, the routine paediatric vaccinations, as well as vaccines for polio. Suitable measures should be taken to ensure the continuity of the different vaccination drives.

6. Awareness campaigns and counselling of people regarding vaccinations should be given an equal priority as they would help in decreasing vaccine hesitancy. The use of digital forums, mainstream and social media should be encouraged for spreading public health awareness.

7. The healthcare infrastructures must be strengthened as co-pandemics can be a serious threat to vulnerable healthcare infrastructures, like those in LDCs and LMICs.

8. The environmental integrity and conservation of biodiversity should be a global priority as it is often zoonoses that are the major drivers of emerging and re-emerging infections. Therefore, concepts like "One Health" should be embraced, and organizations, NGOs and other stakeholders should be encouraged to propagate sustainable and eco-friendly paradigms.

9. A surge in the use of disposable face masks during these unprecedented times are polluting water bodies and becoming a threat to aquatic life. Research into the development of biodegradable facemasks should be supported, and the use of biodegradable facemasks promoted and adopted widely.

10. Robust biosecurity and biosafety structures must be in place. Students and policymakers must be made aware and educated regarding these concepts, which are now considered extremely important amid the pandemic. In this regard, the recently published Tianjin Guidelins for Codes of Conduct for Scientists (https://www.interacademies.org/news/iap-endorses-tianjin-biosecurity-guidelines) can be a useul resource.

11. The use of computer-based technologies, simulations and artificial intelligence, etc. are strongly recommended for the prediction of the trends in infections.

12. Existing knowledge regarding medicinal plants can be used in the search for anti-SARS-COV-2 therapies. Such folkloric knowledge and practices could be used to develop a knowledge base that should be scientifically assessed and verified in anti-viral therapies. However, pseudo-science needs to be discouraged.

13. Tackling misinformation regarding the COVID-19 outbreak is as crucial as searching for a cure. It is critical to tackle falsified or fabricated facts. Advanced IT/computer-aided technologies are required to cope with infodemic situations.

14. Governments should prioritize research and development (R&D), especially in the health sector. Policy-making regarding areas like health, environment, science and technology, etc. should be informed by science and include input from expert scientists in the respective domains.

15. The COVID-19 pandemic has been a source of psychological burden which needs critical attention from scientists, doctors and health workers. Counselling strategies should be developed to cope with the mental health consequences of the pandemic.

16. Public and private research organizations should redouble their commitment to open access to data, knowledge and information especially in the current crisis.

17. Collaborative science between technologically less advanced countries and advanced countries should be encouraged. Such global synergies in the different STEM fields can be helpful in the creation of knowledge as well as in answering pressing healthcare challenges.

18. The COVID-19 pandemic threat is not one of health alone. SDG #2: Zero Hunger and #4: Quality Education, for example, come together when considering the needs of students from marginalized communities in LMICs who may be suffering from the burden of food insecurity while attempting to continue their learning online. Information about physical, mental and healthy coping strategies, as well on affordable, healthy food options is needed.

19. The biggest risk for food security is not considered to be food availability, but rather consumers' access to food. As lockdown measures and other COVID-19-related disruptions lead to a global recession, millions are losing their livelihoods or experiencing a severe drop in income. Social safety nets and food assistance programmes are thus essential to avoid an increase in hunger and food insecurity. Establishing regional genebanks and community seed banks, with safety duplications, can help alleviate some of these issues.

20. Considering virtual education, institutions should apply the following practices to build community and student belonging:

    - Meet students' basic needs;
    - Keep students informed using various communication platforms;
    - Use peer mentors and student leaders to cultivate a sense of community;
    - Provide students with ample opportunities to share their experiences and demonstrate that they are heard by following through with appropriate support;
    - Engage parents and families, providing them with tools and resources to support their students;
    - Increase collaboration to ensure that students are at the centre of all decisions; and
    - Demonstrate care and compassion.

Instructions for Authors

Manuscript Format

The manuscript may contain Abstract, Keywords, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or RESULTS AND DISCUSSION), CONCLUSIONS, ACKNOWLEDGEMENTS, CONFLICT OF INTEREST and REFERENCES, *and any other information that the author(s) may consider necessary*.

Abstract (font size 10; max 250 words)**:** Must be self-explanatory**,** stating the rationale, objective(s), methodology, main results, and conclusions of the study. Abbreviations, if used, must be defined on the first mention in the Abstract as well as in the main text. Abstract of review articles may have a variable format.

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RESULTS: Be clear and concise with the help of appropriate Tables, Figures, and other illustrations. Data should not be repeated in Tables and Figures, but must be supported with statistics.

DISCUSSION: Provide interpretation of the RESULTS in the light of previous relevant studies, citing published references.

ACKNOWLEDGEMENTS: (font size 10)**:** In a brief statement, acknowledge the financial support and other assistance.

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2. W. Bialek, and S. Setayeshgar. Cooperative sensitivity and noise in biochemical signaling. *Physical Review Letters* 100: 258–263 (2008).
3. R.K. Robert, and C.R.L.Thompson. Forming patterns in development without morphogen gradients: differentiation and sorting. *Cold Spring Harbor Perspectives in Biology* 1(6) (2009).
4. D. Fravel. Commercialization and implementation of biocontrol. *Annual Reviews of Phytopathology* 43: 337359 (2005).

b. Books

5. W.R. Luellen. Fine-Tuning Your Writing. *Wise Owl Publishing Company, Madison, WI, USA* (2001).
6. U. Alon, and D.N. Wegner (Ed.). An Introduction to Systems Biology: Design Principles of Biological Circuits. *Chapman & Hall/CRC, Boca Raton, FL, USA* (2006).

c. Book Chapters

7. M.S. Sarnthein, and J.D. Stanford. Basal sauropodomorpha: historical and recent phylogenetic developments. In: The Northern North Atlantic: A Changing Environment. P.R. Schafer, & W. Schluter (Ed.), *Springer, Berlin, Germany*, pp. 365–410 (2000).
8. J.E. Smolen, and L.A. Boxer. Functions of Europhiles. In: Hematology, 4th ed. W.J. Williams., E. Butler and M.A. Litchman (Ed.), *McGraw Hill, New York, USA*, pp. 103–101 (1991).

d. Reports

9. M.D. Sobsey, and F.K. Pfaender. Evaluation of the H2S method for Detection of Fecal Contamination of Drinking Water, Report WHO/SDE/WSH/02.08, *Water Sanitation and Health Programme, WHO, Geneva, Switzerland* (2002).

e. Online references

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10. L. Branston. SENSPOL: Sensors for Monitoring Water Pollution from Contaminated Land, Landfills and Sediment (2000). http://www.cranfield.ac.uk/biotech/senspol/ (accessed 22 July 2005)

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