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Effect of Bio-enhanced *Streptococcus pyogenes* and *Enterococcus faecalis* Co-culture on Decontamination of Heavy Metals Content in Used Lubricating Oil Contaminated Soil

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ABSTRACT

This study assessed the heavy metal decontamination potential of bio-enhanced *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture in used lubricating oil-contaminated soil. The bacterial co-culture was isolated from the soil obtained from Dutse mechanic village, Nigeria. One thousand five hundred(1500) g of sterilized soil was intentionally contaminated with used lubricating oil at three levels. The sterilized soil was biostimulated with processed compost, powdered cocoa pod husk (PCPH), and powdered cattle dung (PCD). Afterward, the mixtures were bio-augmented with the bacterial co-culture (150 mL). The concentrations of Arsenic (As), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Lead (Pb) in the used lubricating oil contaminated soil were determined at the commencement, fifth and tenth week of the study. A factorial experiment which was laid out in a completely randomized design (CRD) was adopted. Results generated from the As decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture indicated that all the organic amendments significantly ($p<0.05$) enhanced its decontamination. At the fifth week, PCPH only enhanced the most Cd decontaminations (0.01020 mg kg^{-1}, 0.00220 mg kg^{-1} and 0.00150 mg kg^{-1}) compared with other organic amendments on 5%, 10% and 15% used lubricating oil contamination levels, respectively. At the tenth week, PCD only enhanced complete removal of Cd on all used lubricating oil contamination levels compared with compost and PCPH only, which attained complete removal of Cd on 5% and 15% of used lubricating oil contamination levels, respectively. The heavy metal decontamination potential of bio-enhanced *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture witnessed in this study indicates its suitability in effecting bioremediation of heavy metal impacted environments.

KEYWORDS: Bacterial co-culture, bioaugmentation, biostimulation, heavy metals, lubricating oil contaminated soil

1. INTRODUCTION

Heavy metal ions derivable from industrial effluents have been implicated as being remarkably toxic even at concentrations that are low. This is largely due to fact that its presence in the environment, even in minute quantities, intolerably disrupts the survival equilibrium of major events in the eco-system, which in turn can be weirdly harmful to human health

(Saravanan et al., 2022). Pollution synonymous with soil is categorized as having the capability of truncating life as all edible plant materials grown therein are invariably eaten by animals and humans (Iyobosa et al., 2020). Numerous authors (Sebiomo et al., 2010; Onuoha et al., 2011; Idemudia et al., 2014; Buraimoh et al., 2017; Ekanem, Ogunjobi, 2017 and Obi et al., 2022) have reported the employment of microorganisms in the reclamation of hydrocarbon impacted media culminating into tremendous success stories over the years.

Effective decontamination of toxic heavy metals by microorganisms can be accomplished through volatilization, extracellular chemical precipitation and valence conversion (Igiri et al., 2018). Bacteria, algae and fungi have the potential to decontaminate toxic heavy metals from impacted environmental media (Wisniewska et al., 2016; Lukic et al., 2016; Kastner and Miltner, 2016; Kanamarlapudi et al., 2018; Adeleye et al., 2019). These authors further reported the use of enzymes, extrusion, manufacture of exopolysaccharide and biotransformation as the mechanisms employed by microorganisms to interact and survive the toxicity of heavy metals.

In Nigeria, mechanic workshops are cited with flagrant contempt for town planning guidelines, thereby giving rise to indiscriminate pollution of the built and natural environments with used lubricating oil emanating from such workshops (Adeleye et al., 2020). According to Kanamarlapudi et al. (2018), toxic heavy metals; Mercury (Hg), Cobalt (Co), Chromium (Cr), Zinc (Zn), Arsenic (As), Copper (Cu), Nickel (Ni), Cadmium (Cd) and Lead (Pb) are inherently found in used lubricating oil. Toxic heavy metals are known not to be biodegradable and can thus accumulate in living organisms' tissues which can, in turn cause numerous human diseases and maladies (Onokebhagbe et al., 2019).

Over the past few years, countless management methods have been employed with a view to removing toxic heavy metals from impacted environments. According to Barakat (2011); Lakherwal (2014); Gunatilake (2015); Azimi et al. (2016); Joshi (2017) and Kanamarlapudi et al. (2018), orthodox methods; microfiltration, ultrafiltration, electro-dialysis, ion-exchange, photocatalysis, chemical precipitation, reverse osmosis, electro-winning, phytoremediation to ultra-filtration were reported to mitigate heavy metal concentration in soil. However, Kanamarlapudi et al. (2018) reported that these remediation methods used for the removal of heavy metals in environmental media are very costly and not eco-friendly. Against these backdrops, this study was conducted to assess the combined effect of bio-enhanced *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture on decontamination of heavy metal content in used lubricating oil contaminated soil.

2. MATERIAL AND METHODS

2.1 Study Area

This study was carried out at the Department of Soil Science, Faculty of Agriculture, Federal University Dutse, Jigawa State, Nigeria. The area lies within Latitude 11.7333N and Longitude 9.2875E (Adeleye et al., 2022a).

2.2 Processing of organic amendments

Processed organic amendments; powdered cattle dung (PCD), powdered cocoa pod husk (PCPH) and compost {fresh cocoa pod husk (CPH) and cattle dung (CD) subjected to composting} employed in this study were produced using procedures earlier outlined by Adeleye et al. (2020). Effective composting of the organic amendments was enhanced through the improved surface area that could enhance faster microbial mineralization by chopping fresh CPH into tiny pieces of less than 5 cm, as outlined by Komolafe et al. (2021).

2.3 Soil collection and processing

Two soils were used in this study. Soil that had no record of pollution (Nkereuwem et al., 2020), was collected from the Teaching and Research farm while used lubricating oil polluted soil was collected from the mechanic village in Dutse town of Jigawa state. Two hundred and fifty-kilogram soils were collected at a depth of 25 cm using a sterilized soil auger through the grid method from four spots in the center of the above-mentioned locations. The uncontaminated soil was simply used to compare the physical and chemical properties of the contaminated soil. The soil was sieved through 2.0 mm mesh size. The soil was there after autoclaved at 121°C for 15 minutes with a view to sterilizing it. One and a half kg of the sterilized soil was subsequently transferred into 36 polyethylene bags each. Three determined levels of used lubricating oil collected from a service pit in Dutse Mechanic Village was added and vigorously mixed with the sterilized soil. Explicitly, these varying levels of the used lubricating oil (75, 150 and 225-mL weight/weight), indicated 5, 10 and 15% contamination levels, correspondingly. The soil-used lubricating oil combinations were left undisturbed for 14 days to ensure the volatilization of the toxic components of the used lubricating oil (Agbor et al.,2015).

2.4 Isolation and identification of *Streptococcus pyogenes* and *Enterococcus faecalis* Co-culture used for heavy metal decontamination

The two bacteria, *Streptococcus pyogenes* and *Enterococcus faecalis* used as a co-culture for the decontamination of heavy metals present in the used lubricating oil contaminated soil in this study were isolated and identified as earlier reported by Adeleye et al. (2022b).

2.5 Experimental design and heavy metal decontamination assay

The design adopted for heavy metal decontamination assay was a 4 × 3 factorial experiment laid out in a completely randomized design (CRD) with three replications. The two factors considered were: (a) organic amendments at four levels, including the control (OA1, having no organic amendment), OA2 representing compost, OA3 representing PCPH, and OA4 representing PCD; and (b) used lubricating oil at three levels of 75 mL representing 5%, 150 mL representing 10% and 225 mL representing 15% contamination levels, labeled ULO1, ULO2 and ULO3, respectively. This experimental layout denotes that *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture (150 mL) were bioaugmented with used lubricating oil contaminated soil in each polyethylene bag except for experimental bags adopted as

control. All the polyethylene bags were then incubated at room temperature for 70 days (Chorom et al., 2010). The contents of each polyethylene bag were subjected to tilling and 6 mL of distilled water was added twice a week for effective aeration and moisture content maintenance, respectively (Abioye et al., 2012).

2.6 Determination of the physicochemical properties of soils and organic amendments

The procedures reported by American Water Works Association (AWWA) (2017); Okareh et al. (2018), were employed to determine available phosphorous and pH in water. Electrical conductivity (EC), moisture content, total nitrogen, organic carbon, texture and other related properties of the soils and organic amendments used in this study were determined using the procedures reported by Adeleye et al.(2020).

2.7 Estimation of heavy metals in the spent engine oil contaminated soil

The procedure reported by APHA (2012) was used to estimate heavy metals; Arsenic (As), Cadmium (Cd), Lead (Pb), Nickel (Ni) and Chromium (Cr) contents in the used lubricating oil contaminated soil at the commencement, 5th week and 10th week of bio-degradation assay using Perkin Elmer Atomic Absorption Spectrophotometer Analyst 400.

2.8 Analysis of Data

All data collected were subjected to analysis of variance using the procedure of the General Linear Model of GenStat Version 17.0. Significant means were subsequently separated using Duncan's new multiple range test at $p< 0.05$.

3. Results and Discussion

3.1 Physicochemical properties of the soil and organic amendments

The results of the physicochemical properties of the soils and organic amendments employed in this study are shown in Table 1.

The pH of the soils employed in this study was acidic (Table 1). It has been reported by Ibrahim et al. (2021) that natural and artificial activities can substantially influence the disparity in the pH of soils. The submission of these authors on African soils being slightly acidic can be substantiated with the current results of the pH recorded in the experimental soils employed in this current study. As witnessed in this study, Osaigbovo et al. (2013) reported that used lubricating oil did contribute significantly to the acidic nature of the soil assayed in their research. The results obtained from the unpolluted soil's mechanical analysis and lubricating oil-contaminated soil indicated them as sandy loam and loamy sand, respectively. The presence of used lubricating oil changed the natural soil texture (sandy loam) of the study area to loamy sand (Table 1). The total sum of exchangeable bases estimated in the used lubricating oil contaminated soil, compost, PCD and PCPH are 1.05, 221.7, 82.1 and 166.15 cmol $kg^{-1,}$ respectively (Table 1).

Table 1. Physicochemical properties of soil and organic amendments

Parameter	US	ULOCS	Compost	PCD	PCPH
Moisture content (%)	2.04	0.8	2.0	7.3	11.11
Ash content (%)	-	-	65	68.8	23
$pH_{(water)}$	6.5	6.8	9.45	8.15	7.6
Organic Carbon (%)	0.49	0.52	48.25	41.55	33.40
Total Nitrogen (%)	0.06	0.08	5.85	2.85	2.65
Available Phosphorous (mg kg^{-1})	11.02	9.40	1.48	1.2	0.08
EC (dS cm^{-1})	0.92	1.20	8.86	8.10	6.42
Exchangeable Bases (cmol kg^{-1})	--	--	--	--	--
Potassium	--	--	--	0.19	0.07
Calcium	--	--	--	1.82	0.63
Magnesium	--	--	--	0.92	0.18
Sodium	--	--	--	0.58	0.17
SEC	--	--	--	3.51	1.05
Particle Size (g kg^{-1})	--	--	--	--	--
Clay + Silt	420	200	--	--	--
Clay	100	120	--	--	--
Silt	320	80	--	--	--
Sand	580	800	--	--	--
Textural class	Sandy Loam	Loamy Sand	--	--	--

Note: US= Unpolluted soil; ULOCS= Used lubricating oil contaminated soil; PCPH= Powdered cocoa pod husk; PCD= Powdered cattle dung; EC= Electrical conductivity; SEC= Sum of Exchangeable Bases; -= Test not conducted

Table 2. Baseline concentrations of heavy metals in used lubricating oil contaminated soil before bioremediation

	Concentrations of heavy metals in used lubricating oil contaminated soil (mg kg^{-1})		
	5%	10%	15%
Arsenic	0.20400	0.20400	0.20400
Cadmium	0.06400	0.06500	0.06400
Chromium	0.3430	0.3460	0.3480
Nickel	0.07100	0.07100	0.08200
Lead	0.6410	0.6390	0.6360

3.2 Baseline concentrations of heavy metals at the commencement of biodegradation assay

Table 2 depicts the baseline concentrations of specific heavy metals estimated in the used lubricating oil contaminated soil based on varying concentrations of the used lubricating oil. It can be seen that As had the same concentration (0.20400 mg kg^{-1}) on 5%, 10%, and 15%, respectively. However, Pb recorded 0.6410 mg kg^{-1}(5%), 0.6390 mg kg^{-1}(10%) and 0.6360 mg kg^{-1}(15%). The presence of the various heavy metals recorded in the used lubricating oil contaminated soil assayed in this current

study had been previously reported by Stephen et al. (2012); Stephen et al. (2013); Zali et al. (2015) and Echiegu et al. (2021) in their respective studies.

3.3 Arsenic decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Results generated from the As decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture indicate that all the organic amendments significantly enhanced its decontamination ($p<0.05$) as shown in Table 3. At the fifth week, compared with other organic amendments, compost recorded the most As removal (0.04130 mg kg^{-1} and 0.04720 mg kg^{-1}) on 5% and 15% used lubricating oil contamination levels, respectively. This finding might be due to the considerable quantities of organic carbon and total nitrogen inherently present in the compost, which aided optimum bacterial growth and metabolic activities (Fadina et al., 2019), thereby leading to significant As decontamination witnessed in this study. However, PCD only recorded the most As decontamination (0.04450 mg kg^{-1}) on 10% used lubricating oil contamination level (Table 3). Interestingly, at the tenth week, all the organic amendments enhanced the complete removal of As on all the used lubricating oil contamination levels (Table 3). Results obtained on As decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture are in line with the report of Bhakta et al. (2014) on *Enterococcus* spp. prowess in the removal of As from water bodies. Similar to this study, Bhattacharyya1 et al. (2013), reported significant detoxification of As by *Streptococcus* spp. isolated from a waste dumping site in India. Bacterial depollution of As in this study corroborates the report of Igiri et al. (2018), on the ability of bacteria to facilitate excellent metal interactions that inevitably lead to its desirable depollution from polluted environments.

3.4 Cadmium decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Results generated from the Cd decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture indicated that all the organic amendments significantly ($p<0.05$) enhanced its decontamination, as shown in Table 4. At the fifth week, PCPH only enhanced the most cadmium removal decontaminations (0.01020 mg kg^{-1}, 0.00220 mg kg^{-1} and 0.00150 mg kg^{-1}) compared with other organic amendments on 5%, 10% and 15% used lubricating oil contamination levels respectively (Table 4). Results generated from the Cd decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture indicated that all the organic amendments significantly ($p<0.05$) enhanced its decontamination, as shown in Table 4. At the fifth week, PCPH only enhanced the most Cd removal decontaminations (0.01020 mg kg^{-1}, 0.00220 mg kg^{-1} and 0.00150 mg kg^{-1}) compared with other organic amendments on 5%, 10% and 15% used lubricating oil contamination levels respectively (Table 4). However, at the tenth week, PCD only enhanced complete removal of Cd on all used lubricating oil contamination levels compared with compost and PCPH only that attained complete removal of Cd on 5% and 15% used lubricating oil contamination levels,

respectively (Table 4). In line with the results obtained in this study, Eghomwanre et al. (2016) reported tolerance of *Streptococcus* spp. isolated from contaminated soils and sediments around the Warri area of Delta State, Nigeria to Cd exposure. Conversely, at the tenth week, PCD only enhanced complete removal of Cd on all used lubricating oil contamination levels compared with compost and PCPH only that attained complete removal of Cd on 5% and 15% used lubricating oil contamination levels, respectively (Table 4). The results are equally in agreement with the report of Bhakta et al. (2014) that implicated *Enterococcus* spp. in the removal of Cd from water bodies. The biostimulatory influence of the organic amendments most especially powdered CD not only enhanced complete bacterial removal of Cd in this study, it also proved influential in attaining such feat owing to the nutrients available therein. The results obtained in this study further corroborate the submission of Huet and Puchooa (2017), on the capability of *Enterococcus* spp. to remove Cd substantially from a medium contaminated with Cd, Pb and Cr.

3.5 Chromium decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Table 5 shows that results generated on the potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture to decontaminate Cr contents of used lubricating oil contaminated soil revealed that the organic amendments employed significantly ($p<0.05$) enhanced such.

At the fifth week, compared with other organic amendments, PCD only enhanced the completed removal of Cr on 5% and 10% used lubricating oil contamination levels, while compost facilitated its complete removal on 15% used lubricating oil contaminated soil (Table 5). At the tenth week, both compost and PCD only enhanced the complete removal of Cr on all the used lubricating oil contamination levels compared with the performance of PCPH (Table 5). The essential nutrients inherently present in the organic amendments employed for biostimulation of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture aided the optimum bacterial metabolism of Cr witnessed in this study. According to Sarkar et al. (2016), the combination of nutrients required for microbial metabolism and growth aids the biodegradation aptitude of the microbial consortium involved in the biodegradation of soils with high concentrations of pollutants. The Cr decontamination attribute that *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture recorded in this study corroborates the submission of Bhattacharyya1 et al. (2013), regarding the substantial Cr detoxification potential of *Streptococcus* spp. isolated from a waste dumping site in their study.

3.6 Nickel decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Results generated on the potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture to decontaminate Ni contents of used lubricating oil contaminated soil reveal that the organic amendments employed did significantly ($p<0.05$) enhance such as shown in Table 6.

Table 3. Interaction of organic amendments and used lubricating oil on bacterial decontamination of Arsenic during biodegradation assay

Treatments		Concentrations of Arsenic (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.20400^{a}	0.20400^{a}
OA1	ULO2	0.20400^{a}	0.20400^{a}
OA1	ULO3	0.20400^{a}	0.20400^{a}
OA2	ULO1	0.04130^{i}	0.00000^{b}
OA2	ULO2	0.04453^{g}	0.00000^{b}
OA2	ULO3	0.04720^{f}	0.00000^{b}
OA3	ULO1	0.07370^{d}	0.00000^{b}
OA3	ULO2	0.07770^{c}	0.00000^{b}
OA3	ULO3	0.08120^{b}	0.00000^{b}
OA4	ULO1	0.04240^{h}	0.00000^{b}
OA4	ULO2	0.04450^{g}	0.00000^{b}
OA4	ULO3	0.04920^{e}	0.00000^{b}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range test (DMRT). ($p>0.05$) 0.00000= Not detected

Table 4. Interaction of organic amendments and used lubricating oil on bacterial decontamination of cadmium during biodegradation assay

Treatments		Concentrations of Cadmium (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.06400^{b}	0.06400^{b}
OA1	ULO2	0.06500^{a}	0.06500^{a}
OA1	ULO3	0.06400^{b}	0.06400^{b}
OA2	ULO1	0.01540^{g}	0.00000^{g}
OA2	ULO2	0.02090^{e}	0.00090^{e}
OA2	ULO3	0.03750^{c}	0.01150^{c}
OA3	ULO1	0.01020^{i}	0.00020^{f}
OA3	ULO2	0.00220^{j}	0.00120^{d}
OA3	ULO3	0.00150^{k}	0.00000^{g}
OA4	ULO1	0.01340^{h}	0.00000^{g}
OA4	ULO2	0.01760^{f}	0.00000^{g}
OA4	ULO3	0.02400^{d}	0.00000^{g}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range test (DMRT). ($p>0.05$) 0.00000= Not detected

Table 5. Interaction of organic amendments and used lubricating oil on bacterial decontamination of Chromium during biodegradation assay

Treatments		Concentrations of Chromium (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.3430^{c}	0.3430^{c}
OA1	ULO2	0.3460^{b}	0.3460^{b}
OA1	ULO3	0.3480^{a}	0.3480^{a}
OA2	ULO1	0.0540^{h}	0.0000^{g}
OA2	ULO2	0.0450^{i}	0.0000^{g}
OA2	ULO3	0.0000^{j}	0.0000^{g}
OA3	ULO1	0.3120^{d}	0.2130^{d}
OA3	ULO2	0.3010^{e}	0.2020^{e}
OA3	ULO3	0.1950^{f}	0.1130^{f}
OA4	ULO1	0.0000^{j}	0.0000^{g}
OA4	ULO2	0.0000^{j}	0.0000^{g}
OA4	ULO3	0.0850^{g}	0.0000^{g}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range test (DMRT). ($p>0.05$) 0.0000= Not detected.

Table 6. Interaction of organic amendments and used lubricating oil on bacterial decontamination of Nickel during biodegradation assay

Treatments		Concentrations of Nickel (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.07100^{b}	0.07100^{b}
OA1	ULO2	0.07100^{b}	0.07090^{c}
OA1	ULO3	0.08200^{a}	0.08150^{a}
OA2	ULO1	0.04230^{h}	0.02223^{l}
OA2	ULO2	0.04407^{gh}	0.02920^{k}
OA2	ULO3	0.05647^{cd}	0.03690^{h}
OA3	ULO1	0.05160^{def}	0.05020^{f}
OA3	ULO2	0.05320^{de}	0.05150^{e}
OA3	ULO3	0.06130^{c}	0.05620^{d}
OA4	ULO1	0.04663^{fgh}	0.03160^{j}
OA4	ULO2	0.04880^{efg}	0.03670^{i}
OA4	ULO3	0.05450^{de}	0.04930^{g}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range test (DMRT). ($p>0.05$)

Table 7. Interaction of organic amendments and used lubricating oilon bacterial decontamination of Lead during biodegradation assay

Treatments		Concentrations of Lead (mg kg^{-1})	
OA	ULO level	5th Week	10th Week
OA1	ULO1	0.6410^{a}	0.6410^{a}
OA1	ULO2	0.6390^{b}	0.6390^{b}
OA1	ULO3	0.6360^{c}	0.6360^{c}
OA2	ULO1	0.2260^{i}	0.0027^{k}
OA2	ULO2	0.1470^{j}	0.0080^{j}
OA2	ULO3	0.0680^{k}	0.0380^{g}
OA3	ULO1	0.3180^{g}	0.1160^{e}
OA3	ULO2	0.0110^{l}	0.0000^{l}
OA3	ULO3	0.2730^{h}	0.1230^{d}
OA4	ULO1	0.3880^{e}	0.0130^{i}
OA4	ULO2	0.3570^{f}	0.0260^{h}
OA4	ULO3	0.3990^{d}	0.0430^{f}

Note: OA= Organic amendment; ULO= Used lubricating oil; OA1= without organic amendment; OA2= Compost; OA3= Powdered cocoa pod husk only; OA4= Powdered cattle dung only; ULO1= ULO at 5%; ULO2= ULO at 10%; ULO3= Used lubricating oil at 15%. Means with the same letter (s) in each column are not significantly different using Duncan multiple range tests (DMRT). ($p>0.05$) 0.0000= Not detected

However, at the fifth week, compared with other organic amendments, compost influenced the most reductions (0.04230 mg kg^{-1}and 0.04407 mg kg^{-1}) on 5% and 10% used lubricating oil contamination levels, respectively, while PCD only enhanced the most reduction (0.05450 mg kg^{-1}) on 15%used lubricating oil contamination level (Table 6). At the tenth week, compost significantly ($p<0.05$) enhanced the most Ni decontamination (0.02223 mg kg^{-1}, 0.02920 mg kg^{-1} and 0.03690 mg kg^{-1}) compared with other organic amendments on 5%, 10% and 15% used lubricating oil contamination levels respectively (Table 6). These results have shown that organic nutrient supplementation can enhance bacterial decontamination of Ni when present in an undesirable concentration in the environment. The bacterial decontamination of Ni in this study is in agreement with the report of Alfano and Cavazza (2020) on its importance in redox processes and microbial metabolism.

3.7 Lead decontamination potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture

Results generated on the potential of *Streptococcus pyogenes* and *Enterococcus faecalis* co-culture to decontaminate Pb contents of used lubricating oil contaminated soil revealed that all the organic amendments engaged significantly enhanced such ($p<0.05$) as shown in Table 7. Specifically, at the fifth week, compared with other organic amendments, compost influenced the most reductions (0.2260 mg kg^{-1} and 0.0680 mg kg^{-1}) on 5% and 15% used lubricating oil contamination levels, respectively while PCPH only enhanced the most decontamination (0.0110 mg kg^{-1}) of Pb on 10% used lubricating oil contamination level (Table 7).

At the tenth week, compared with other organic amendments employed, compost further significantly ($p<0.05$) enhanced the most reductions (0.0027 mg kg^{-1} and 0.0380 mg kg^{-1}) on 5% and 15% used lubricating oil contamination levels, respectively while PCPH only facilitated complete removal of Pb on 10% used lubricating oil contamination level (Table 7). The feat that the bacterial co-culture attained in terms of tolerance of Pb and its eventual decontamination from the used lubricating oil contaminated soil studied can be attributed to its effective supplementation with suitable nutrients that ultimately enhanced its performance. Similar finding has been reported by Adeleye et al. (2020) on the ability of effective supplementation of bacteria with organic amendments leading to significant decontamination of heavy metals from used lubricating oil contaminated soil. These results further support the report of Huet and Puchooa (2017), on the proficiency of *Enterococcus* spp. in recording the highest level of Pb removal in their study.

4. CONCLUSION

Bacterial decontamination of the heavy metal contents of varying levels of used lubricating oil contaminated soil was significantly enhanced through biostimulation with processed organic amendments in this study. However, compared with the controls employed, which recorded poor heavy metal decontamination, the concentrations of heavy metals decontaminated were significantly higher in the bio-enhanced used lubricating oil contaminated soil samples. Resultantly, the blend of bio-augmentation and bio-stimulation technologies is recommended for the clean-up of heavy metal impacted environments due to the significant removal of such recorded in this study.

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A.O.A. and M.B.Y. conceptualized the research. M.E.N and V.O.O.were actively involved in the physicochemical analysis of the soils and biostimulants. M.E.N, analyzed the data generated from the research. A.O.A. wrote the manuscript. M.B.Y and M.G.D. revised the manuscript.

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Mini Review

Response of Soil Proteobacteria to Biochar Amendment in Sustainable Agriculture- A mini review

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ABSTRACT: In recent years, biochar application to soil has become more popularized due to its potential roles on soil fertility, plant growth, and development. In this review, we discussed the impact of biochar on the relative abundance of soil proteobacteria and its relationship with soil physiochemical properties under different rhizospheres. It was observed that biochar applied to different soil improved proteobacteria, and its lowest and highest relative abundance was ranged from 30-80%, respectively. A positive relationship of soil proteobacteria with soil pH, total nitrogen, available phosphorous, available potassium and total carbon were observed in several studies. Both the relative abundance of proteobacteria and its relationship with soil properties depend on biochar type, soil type, and fertilizers applied to the soil. Most of the ammonia-oxidizing bacteria including nitrogen-fixing bacteria, ammonia-oxidizing bacteria, cellulose-decomposing bacteria, nitrifying bacteria and denitrifying bacteria belong to proteobacteria, which plays a significant role in nitrogen recycling that is beneficial for the plant growth, yield and fruits/seeds quality. Furthermore, a positive relationship between soil proteobacteria and plant yield was also highlighted. In this context, the use of biochar play a potential role to improve the relative abundance of proteobacteria in sustainable agriculture. We highlighted future research guidelines that might benefit the sustainable agricultural system. Moreover, further studies are needed to explore the potential role of biochar application on Proteobaceria families such as *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria,* and *Epsilonproteobacteria*.

KEYWORDS: Biochar, soil proteobacteria, soil properties, different rhizospheres, relationships

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1. Introduction

Soil microbes play a vital role in the ecological processes of soil containing organic matter decomposition and soil aggregates formation (Condron et al., 2010). This not only increases the soil fertility, but also enhance a series of ecosystems (Lemanceau et al., 2015). Several studies have reported an increase in soil microbes diversity and biomass with the amendment of biochar to soil (Dangi et al., 2020; Diacono et al., 2011; Han et al., 2010; Meng et al., 2019). Meng et al. (2019) observed that wheat straw-derived biochar amendment to soil

significantly increased the abundance and diversity of plants beneficial bacteria in the rhizosphere of wheat seedlings. Among soil bacteria's, proteobacteria is the largest phylum of abundant bacteria, which are composed of mesophilic and neutrophilic bacteria (Fukuyama et al., 2010), and are related to a wide range of functions involved in carbon, nitrogen, and sulphur cycling (Mhete et al., 2020). Previous research studies have reported that nitrogen-fixing bacteria, ammonia-oxidizing bacteria, cellulose-decomposing bacteria, nitrifying bacteria and denitrifying bacteria are a significant effects on the nitrogen cycle, and most ammonia-oxidizing bacteria belong to proteobacteria (Liang et al., 2012; Stein et al., 2003). Proteobacteria members are predominant in several soil ecosystems, including the rhizospheres, saline soil and semiarid soil (Mhete et al., 2020). Kersters et al. (2006) reported that proteobacteria consists of more than 460 genera and more than 1600 species, scattered over 5 major phylogenetic lines of descent known as the classes Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria.

Improving the production of crops in modern agriculture increased the uses of fertilizer (Ali et al., 2020). Thus, the overuse of fertilizers can directly influence the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil (Nakhro et al., 2010). Biochar (BC) is a carbon-rich, stable product that is produced by the burning of organic material (biomass) of agricultural and forestry wastes via a controlled process called pyrolysis, and is well known for improving crop production and soil health (Ali at al., 2020; Ullah et al., 2021; Ali et al., 2022, Khan et al., 2021). Positive or negative effects of BC on total soil microbial community as well documented (Khan et al., 2014; McElligott et al., 2011; Nguyen et al., 2018). According to some reports, application of biochar to soil may provide a direct threat to soil flora and fauna, but it may also promote soil health, significantly change the make up of the soil biological community, and boost soil microbial biomass (Ullah et al., 2021; Liang et al., 2010; O'Neill et al., 2009; Jin, 2010). Meanwhile, due to the sensitivity of soil microbes, application of biochar to soil may have an impact on soil microbial populations, community structure, and physiological activities (Dempster et al., 2012; Dai et al., 2016; Lehmann et al., 2011). It has been noted that soil microbial abundance rose proportionally as biochar production rate improved (Gomez et al., 2014), As opposed to this, Ameloot et al. (2014) reported the opposite effects, claiming that 49 t biochar per ha introverted microbial activity and condensed both extractable phospholipids (PLFA) concentration and fungal abundance. Furthermore, (Ali et al., 2020; Han et al., 2017) reported that biochar addition to soil can considerably improve the soil pH, which potentially provides a more favorable habitat for microbial organisms, especially bacteria that are sensitive to pH (Han et al., 2017).

As a result, the application of biochar alteration in soil biological processes is a new field of study for soil science, and there are many anomalies that need to be explored. Recently many reviews have summarized the responses of soil biota and fertility to biochar

amendment (Lehmann et al., 2011; Ding et al., 2016), providing a good description of biochar application and soil microbial community composition. To date, no review is published that included detail of a single bacteria "proteobacteria" relative abundance in response to biochar amendment. Therefore, improvements in our understanding of biochar necessitate a review of its impacts on the abundance of soil proteobacteriaand its relationship to soil properties to develop a road map for future research.

2. Changes in the relative abundance of proteobacteria in sustainable agriculture

Fertilizers are essential for better crop growth, yield and grain quality. Previously, it was reported that diverse fertilizers amendment can alter soil proteobacteria, for example, nitrogen application is increases for long term (Dai et al., 2018) or decreases (Kalivas et al., 2017) the relative abundance of proteobacteria. Another study analyzed the dominant microbiome at the phylum level and their results showed that the dominant phyla were proteobacteria ranging the relative abundance from 51 to 58% (Li et al., 2020). The negative impact of NPK fertilizer on soil proteobacteria were also reported by Soni et al. (2016) and Shao et al. (2018), and their results suggested that the relative abundance of specific bacterial phyla was influenced by soil chemical (salinity) and biochemical properties. Further, Liang et al. (2020) documented that the relative abundance of proteobacteria was significantly higher in bio-organic fertilizer treatments as compared to nitrogen fertilizer applied treatments (Table 1; Figure 1).

In contrast, Gu et al. (2020) concluded that nitrogen application increased the relative abundance of proteobacteria compared to control treatment. They highlighted that available nitrogen was the main factor, which may influence these organisms in the soil. However, 25 - 45% of the relative abundance of proteobacteria was found in grape rhizosphere treated with typical chemical fertilization for three consecutive years, which might due to containing more available N and available P than the rhizosphere soil of the other treatments (Wu et al., 2020). In the grassland rhizosphere treated with mushroom residue, the relative abundance of proteobacteria was recorded, ranging from 22-23% as compared to control treatment (Shang et al 2020). Their results are in agreement with previous studies on bacterial community composition in grassland soil (Chen et al., 2017; Xu et al., 2018; Nacke et al., 2011). Sun et al. (2019) reported that proteobacteria relative abundance ranges about 58% in grassland with nitrogen fertilizer application.

Furthermore, the classes of proteobacteria, including *Alphaproteobacteria* and gamma proteobacteria, were increased in 269 kg N ha^{-1} compared to the control treatment (Sun et al., 2019). *Betaprotobacteria* and *Deltaproteobacteria* decrease and were not significantly affected by nitrogen fertilizer (Sun et al., 2019). Li et al. (2021) reported that nitrogen fertilizer under different irrigation levels improves substantially the abundance of proteobacteria followed by Bacteroidetes Actinobacteria, Acidobacteria. The range of proteobacteria abundance was recorded from 28.46–37.78% t 120 kg N ha^{-1} in the wheat field (Li et al., 2021).

Table 1. The relative abundance of soil proteobacteria in different rhizosphere and different fertilizers applications.

Rhizosphere	Proteobacteria relative abundance	Fertilizer used	References
Maize	51.17–57.89%	NKP	Li et al. (2020)
Wheat	29.67%–34.15%	Bio-organic fertilizer	Liang et al. (2020)
Sugarcane	31.23–40.68%	N375 and N563 kg ha^{-1}	Gu et al. (2021)
Grape	25-35%	NPK fertilization for three consecutive years	Wu et al. (2020)
Hulunbuir Grassland Ecosystem	22-23%	Organic fertilizer	Shang et al. (2020)
Grass land	58%	Nitrogen Fertilizer	Sun et al. (2019)
Sugarcane	45%	NPK	Khan et al. (2021)
Tomato	86-89%	Chicken manure	Haq et al. (2021)
Wheat	28.46–37.78%	N fertilizers	Li et al. (2021)
Maize	23.2%	N fertilizer	Muhammad et al. (2022)
Sugarcane	47%	NPK	Khan et al. (2022)
Rice	30-40%	Manure + NPK	Iqbal et al. (2022)

In addition, a recent study reported that N fertilizer at the rate of 300 kg ha^{-1} under different irrigation levels increased proteobacteria and was the most abundant bacteria, ranging by 23%, followed by Firmicutes (Muhammad et al., 2022). Similarly, Khan et al. (2022) reported that nearly 47% of the total species found in the rhizosphere of ratoon crops were proteobacteria, nevertheless, the fraction of several bacterial species changed during the second rationing.

Several studies reported the relationships among soil properties with environmental factors and proteobacteria. The positive relationship of proteobacteria with soil P, N-NH_4, NO_3-NO_2-N, Nt, Ct, OM, and moisture was recorded in grassland soil treated with NPK fertilizers (Pan et al., 2014). Khan et al. (2021) evaluated the response of bacteria at the phylum level in the sugarcane rhizosphere, and they observed 45% of relative abundance of proteobacteria at phylum level, whereas at class level Gamma-proteobacteria were the highest by 30% of relative abundance. Meanwhile, proteobacteria in soil were found positively correlated to the metal nutrients arability, especially to calcium (16 OTUs) and magnesium (19 OTUs) (Zhang et al., 2020). Haq et al. (2021) determined that the relative abundance of proteobacteria was higher in the soil treated with chicken manure ranging from 86-89% in the tomato rhizosphere as compared to control.

The changes in soil proteobacteria are mostly attributed to the status of nutrients available in the soil. Likewise, numerous studies observed that the abundance of soil proteobacteria was significantly influenced by soil physiochemical properties (Li et al., 2020; Liang et al., 2020; Gu et al., 2021; Wu et al., 2020; Shang et al., 2020; Sun et al., 2019; Khan et al., 2021). Currently, improving soil physiochemical properties and biochemical properties facing a great challenge, around the globe agronomist suggested the use of biochar in sustainable agriculture to improve soil health and crop production (Ali et al., 2020, Ullah et al., 2021; Ali et al., 2021; Khan et al., 2021; Ding et al., 2016; Diatta et al., 2016; Imran, 2021). Numerous studies focused as whole soil microbe's responses to biochar in different rhizosphere, we reviewed the article on the plant growth promoting bacteria "proteobacteria" in response to biochar to understand the role of biochar in relation to soil most abundant bacteria.

3. Biochar and soil proteobacteria

The use of biochar as a targeted method for managing soil biota is gaining popularity; while in advertent changes in soil biota as a result of biochar use are also strong concerns. This is an essential area of research because the health and diversity of soil bacterial populations are important for soil function and ecosystem services, which in turn affect soil structure and stability, nutrient cycling, aeration, water use efficiency etc. (Table 2) (Khan et al., 2021). Therefore, we assumed to review the relative abundance of soil proteobacteria under different biochar amendment in different rhizospheres. Results showed that soil proteobacteria was found the most abundant bacterium in response to biochar treatments as shown in figure 1. Moreover, Yin et al. (2021) reported that compared to control, corn stalk biochar application increased the relative abundance of proteobacteria by 13% in rice rhizosphere under pot experiment. This is because proteobacteria is a eutrophic bacterium, and biochar addition has been proven to improve the nutritional properties of albic soils (Fierer et al., 2007), resulting in an increase in proteobacteria abundance.

Fan et al. (2020) investigated that bacterial community composition after six years of biochar addition, and proteobacteria was the most abundant phyla accounting for 39.0–40.4% of the total composition. However, in their study biochar did not change the abundance of proteobacteria compared to control, while the relative abundance of Nitrospirae and Verrucomicrobia phylum increased but that of Acidobacteria phylum decreased significantly in biochar amended soils. Similarly, Kong et al. (2020) recorded proteobacteria as most abundant phyla ranging from 48.26–82.32% in mountain soil treated with ground residue biochar. In contrast, Liao et al. (2019) documented that biochar addition to soil decreased the abundance of proteobacteria. Similarly, another study also found that the relative abundance of proteobacteria was much higher in the control treatment as compared to biochar treatments (Kolton et al., 2011). Besides, Zhang et al. (2020) reported that the relative abundance of proteobacteria was

Table 2. Response of proteobacteria to different biochar's in various rhizospheres.

Biochar type	Proteobacteria (Total relative abundance)	Rhizosphere	References
Rice straw and corn straw biochar	30.69-34.97%	Rice	Yin et al. (2021)
Corn straw at a pyrolysis temperature of 500 ◦C	39-40%	Rice	Fan et al. (2020)
Ground residue at a pyrolysis temperature of 500 ◦C	48.26–82.32%	Mountain	Kong et al. (2020)
Rice straw 500 ℃,	35.45%	Tobacco fields	Zheng et al. (2021)
Cpple (Maluspumila Mill.) wood chip pyrolyzed at 500°C	43 and 35%	Intercropping of bean (Viciafaba L.), and a cereal crop, maize (Zea mays L.),	Liao et al. (2019)
Citrus wood	71 to 47%.	Pepper Plants	Kolton et al. (2011)
Maize straw (600ºC)	73.54%±3.11	Sea grass	Zhang et al.(2020)
Cassava straw (400-500 ºC)	30-50%	Rice	Ali et al. (2022)
Sewage sludge biochar (300-600 ºC)	20-45%	Agriculture field soil	Ahmad et al. (2022)
Weed, Ageratina adenophora (Spreng.) (500%)	24-72%	Cucumber	Li et al. (2022)
Cotton straw biochar (450 °C)	90-96%	Cotton field Soil	Zhu et al.(2022a)
Rice Straw (500-600°C)	21%	Pomelo Orchard	Song et al.(2022)
Fruit tree residues (550 °C)	87.8–88.9%	Experimental Field (Clay soil)	Zhang et al. (2022b)
Rice straw (450 ◦C)	22.53%	Mountain Watershed	Wang et al. (2022)
Bark of Italian poplar (600℃)	30-50%	Polyethylene flowerpot	Zhu et al. (2022b)

found most abundant 73.54%±3.11 for rhizosphere sediment under biochar application. In case of class level, on day 14 for sediment bacterial communities, *Alphaproteobacteria* with biochar addition treatments had a decreased in relative abundance, whereas *Gammaproteobacteria* had a higher relative abundance. (Zhang et al., 2020a). Zhang et al. (2022b) observed proteobacteria, Cyanobacteria, and Actinoba cteria the most dominant phyla in clay treated with fruit tree residues biochar. Furthermore, they attributed that changes in microbial diversity was due to soil porosity, soil moisture content, organic matter N and P fertilizer(Zhang et al., 2022b).

After three years of biochar applied improved soil physiochemical properties which consequently improved soil proteobacteria accounted for 30-50% of total bacterial abundance under filed condition (Ali et al., 2022). They observed that changes in bacterial abundance were strongly dependent on soil physiochemical properties. Recent research recorded that phyla proteobacteria was the most abundant bacteria and accounted for 40-50% of the total bacterial population in soil treated with biochar which can enhance the soil nutrient cycle (Ahmad et al., 2022). They observed that different pyrolysis temperature of biochar significantly affected soil bacterial community structure. However, an increase in soil enzymatic actives and a decrease in proteobacteria-relative abundance were recorded in soil treated with biochar applied compared to control and compost treated soil (Azeem et al., 2020). A higher range of 24 -72% of relative abundance of proteobacteria in cucumber under pot experiment condition was recorded by Li et al. (2022). They further documented that the bulk soil bacterial populations did not change considerably or even reduced, the relative abundance of proteobacteria and Actinobacteria grew dramatically in the rhizobacterial communities. Similarly, 90-96% of proteobacteria relative abundance was recorded in soil treated with cotton biochar in ceramic pots (Zhu et al., 2022). They further observed that proteobacteria relative abundances was increased by 11.58% in biochar treatment.

4. Relationship of soil proteobacteria with soil properties under biochar application

Soil physiochemical properties play a vital role in soil microbial abundance including soil proteobacteria. While, biochar is a key factor of changing soil physical and chemical properties (Ali et al., 2020; Ullah et al., 2021; Ali et al., 2021). An earlier study found that the first axis of soil microbial community composition is considerably impacted, either favorably or negatively, by soil pH, TN, C/N, TC, AK, TK, and AN (Fan et al., 2020).

Fan et al. (2020) reported that the abundance of proteobacteria was significantly positive associated with soil pH, TC, TN AK and AP, while it's was negatively correlated with TK and AN under biochar application. Which indicates that biochar application speckled bacterial community composition indirectly through variation in soil properties. A negative correlation between soil pH, MBN and MBC with soil proteobacteria was observed by Kong et al. (2021). However, under NPK fertilization, Khan et al. (2021) reported a positive correlation of soil proteobacteria with soil N, P and K in sugarcane rhizosphere along with different

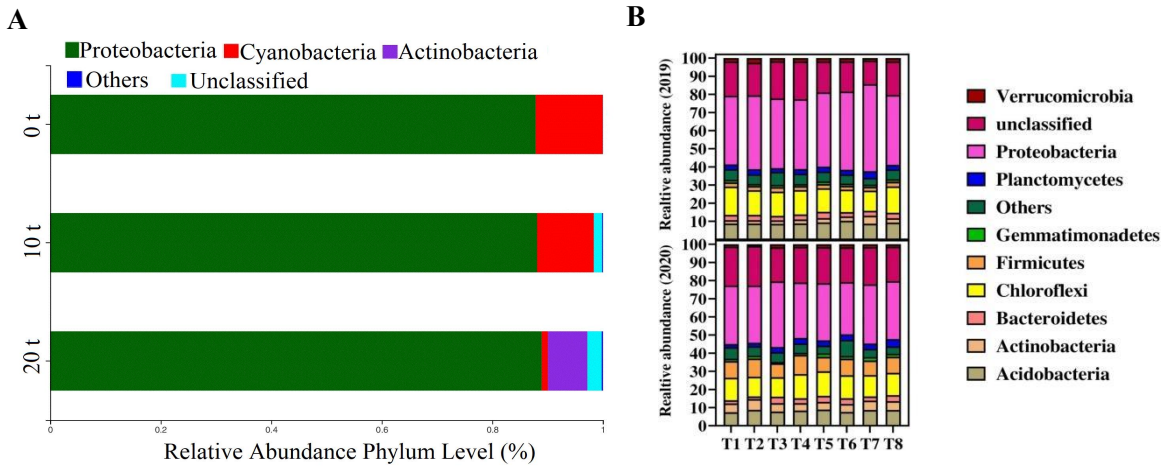

Figure 1. Relative abundance of dominant bacterium with biochar addition over 45 days incubation applied treatments (A) (Sun et al., 2021) and after two years in rice field (B) (Ali et al., 2022).

cultivars in Guangxi Province of China. A positive relationship of relative abundance of proteobacteria with soil pH, moisture, AP, and AK were recorded under different nitrogen and irrigation levels (Li et al., 2021). However, a negative relationship with soil pH, and positive relationship with soil enzymatic activities with bacterial phyla under N and irrigation levels was reported by Muhammad et al. (2022). According to Ali et al. (2022), proteobacteria (R=0.32) have a substantial positive correlation with paddy rice grain yield, whereas Chloroflexi, Firmicutes, Gemmatimonadetes, and Verrucomicrobia have no visible relation with rice grain yield. Furthermore, another finding revealed a close correlation between the relative abundances of Acido-bacteria and proteobacteria and the soil biochemical characteristics (pH, C/N ratio, and soil enzyme activity), suggesting that the soil biochemical characteristics had a significant impact on the relative abundances of Acidobacteria and proteobacteria (Zhu et al., 2022).

5. Mechanism of biochar to improve soil proteobacteria

Biochar application is well-known to improve soil health, including enzymatic activities, physiochemical properties, microbial biomass carbon and nitrogen. These factors are directly or indirectly correlated with soil bacterial abundance and composition structure and proteobacteria accounted in the top abundant bacteria's. Based on these findings, a hypothetical mechanism of biochar was proposed affecting soil proteobacteria abundance. Firstly, biochar application could consistently improve soil pH (Ali et al., 2020; Ullah et al., 2020), total nitrogen (Son et al., 2022; Ullah et al., 2021a), available phosphorous (Tesfaye et al., 2021; Ullah et al., 2021b), available potassium (Wang et al., 2018), total carbon (Ali et al., 2021).

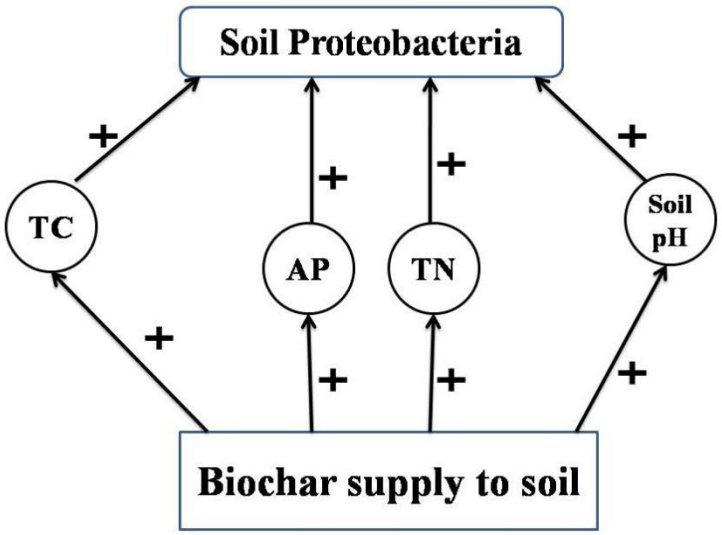

Figure 2. A Mechanism involving the soil properties has an influence on soil proteobacteria abundance under biochar. (+) and (-) represented increased and decreased effects, respectively. Note; TC-total carbon, AP-available phosphorous, TN-total nitrogen,

Secondly, these soil indicators were significantly positively correlated with soil bacterial abundance and especially proteobacteria (Ali et al., 2022; Song et al., 2022; Khan et al., 2022). In other words, the model has highlighted that the soil physiochemical properties could positively affect soil proteobacteria under biochar applied treatments. In addition, since biochar could distinctively alter soil proteobacteria, it remains interesting to explore whether it also affects Proteobaceria families such as *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria,* and *Epsilonproteobacteria*.

6. Conclusion, limitations and future aspects

The available literature provides ample justification for further investigation into the effects of biochar on proteobacteria, which contributes to soil fertility indirectly through ammonia-oxidizing bacteria. The abundance of this bacterium significantly influences plant roots, growth and yield. A great abundance of proteobacteria in soil with biochar application is relatively well established (Table 2; Figure 2). Apart from using biochar as inoculants carriers, little information about using biochar to manage proteobacteria on class, order, family, genus and species level. The knowledge gap needed an urgent attention including biochar effects on ammonia-oxidizing bacteria including nitrogen-fixing bacteria, ammonia-oxidizing bacteria, cellulose-decomposing bacteria, nitrifying bacteria and denitrifying bacteria belongs which belongs to proteobacteria. Furthermore, the effect of biochar under different rhizosphere on proteobacteria family such as *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*, *Deltaproteob-*

acteria and *Epsilonproteobacteria* need to be documented. Important questions emerged from a biochar-sphere perspective: How far does the influence of biochar reach into different soil *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*, *Deltaproteobacteria* and *Epsilon proteobacteria* abundances? What are the critical soil physiochemical properties influences proteobacteria species?

Authors Contributions:

Z. H and M.A conceived the main idea of research, Z.H and F.U., wrote the review article. S.U.A.S., R.A, M.A, F.U., A.K., and Q.H revised the draft and provided suggestions. All authors have read and agreed to the published version of the review article.

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Review

Comprehensive analysis of the mechanism underlying plastic microbiome and plants interaction, with future perspectives

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ABSTRACT: Agriculture has a vital role in the life cycle of an economy. Phytopathogenic microorganisms negatively influence many crops, the economy, and the Environment worldwide. Beneficial plant microbiomes have the immense potential to provide cost-effective and maintainable solutions to existing agricultural challenges. The yield improvement can partly be credited to advanced plant pest and disease management, including better knowledge of phytopathogens and diverse control methods. Well-organized and balanced crop protection is of vast economic and ecological importance for food and feed production. A varied variety of goods made of plastics are utilized in farming which consists of poly-tunnels, plastic reservoirs, mulches, ropes, agrochemical cans, various nets, irrigation systems, packaging bags, nursery pots, anti-bird nets, greenhouses, and their components, wear and tear of these products are hosts of diverse microorganisms in agriculture. However, little investigation has been done to explore plastic microbes' diversity, survival strategies, and interaction mechanisms with plants. Several advanced approaches, including metagenomics, metabolomics, metatranscriptomics, metaproteomics, and culturomics, are currently available to scrutinize the multiplicity, composition, and functions of the microbiomes in soil and plant habitats such as rhizosphere, phyllosphere, and endosphere. This review highlights the increasing use of plastic, plastic microbiomes, subsequent challenges, and future perspectives in agriculture. It emphasizes using advanced molecular tools and techniques to explore the microbiome diversity and the mechanism of plant-microbe interaction. The analyzed knowledge gaps in the host-pathogen relationship research area will help to redraft better research approaches based on economic thresholds.

KEYWORDS: Agricultural plastic, plant microbiome interaction, agrotechnical control, omics application, competence and persistence

1. Introduction

As sessile organisms, plants are under endless attack by microbes entering them for nutrients, shelter, and survival. Mutualistic and parasitic microorganisms colonize the above and below-ground parts of the plant. These microbes could be divided into two groups according to colonization areas, those which inhabit the exterior parts of the plant, generally called epiphytes, and those that live inside the plants are endophytes. Phyllosphere microorganisms colonize the leaf surface, and those which populate regions closest to the root system are the most abundant microorganisms, known as rhizosphere inhabitants (Dong et al., 2019; Sivakumar et

al., 2020). The rhizosphere microbes are attracted to the root system because of root exudates (Lakshmanan et al., 2014). The exudates differ according to the plant's developmental stages and physiological conditions (Choudhary et al., 2016). The conscription of microbes to the root regions results from plant exudation and has a diverse role in the growth, development, and inhibition of host pathogens (Philippot et al., 2013). This association suggests an interdependency between the host plants and microbes in the above and below-ground environments.

Sustainability is the capability of the ecosphere and human society to live together. Three main factors compose sustainability: society, Environment, and economy (Naamala et al., 2016). So, it is significant to scheme economically affordable mechanisms to implement approaches to protect resources, be Environment friendly, and have social acceptance. Knowing enough about the tools and pathways of critical values necessary for conniving strategies to face increasing global food demand without destroying the ecosystem is essential. Diverse microorganisms in contact with plants consist of organisms living on surfaces, within cells, and in the soil near the root interfaces of the plant (Cepeda, 2012).

Various microbial groups impact plant health, growth, development, and yield positively or negatively. Indirectly, microbes could become a source of negativity, such as the initiation of diseases in plants, the manufacture of phytotoxic elements, the encouragement of soil-borne microorganisms, and the reticence of seed germination (Daisley et al., 2022). The existence of particular pathogens in agriculture can bring complete crop failure. If the situation is so, the farmers need blanket approaches to extinguish harmful microbes' threats (Watson et al., 2002).

Beneficial microorganisms have numerous roles and actions within the agricultural sector that are significant for plant sustainable growth and development (Sundh et al., 2021). Plant growth-promoting bacteria have been reported to play an essential role in maintaining the soil's physical, chemical, and biological properties by accelerating nutrient and water uptake, particularly in abiotic stress situations, one of the main hurdles in agricultural development and yield improvement (Abdelaal et al., 2021). Unearthing novel microorganisms and assessing their efficiency would be significant for high-yield sustainability in agriculture (Herman et al., 2019).

2. Plants microbe interaction

During their life, plants continuously interact with diverse microorganisms, such as pathogens, symbionts, and commensal. These microbes' interactions could significantly impact plants' vital traits, including their growth and resilience against biotic and abiotic stress and shelf life. Pathological microbes severely threaten the growth and yield of essential crop plants worldwide. However, the interaction between plants and microbes may have positive or negative effects according to the types and nature of plants and microbes and their circumstances (Compant et al., 2019). The genes of microbes in the microbiome that interact with plants have their genetic

repertoire and can improve the host plant's adaptation to an environmental perturbation or prevent it from performing (Douglas and Werren, 2016; López-Mondéjar et al., 2017). So, it needs a meticulous investigation to isolate the beneficial microbes from microbiomes and use them for crop growth improvement, yield enhancement, and cost reduction.

3. Sources of microbial contamination

3.1 Plastic as microorganisms host

Food, fruits and vegetables have significant nutritional values and health impacts established for a long time. Plants, mainly comestible vegetables, and other crops host various microorganisms on their surfaces. Some recognized bacterial contamination sources include contaminated manure, irrigation water, soil, livestock, wildlife, and several other factors, mainly plastics, that cause the occurrence, destiny, transportation, survival, and proliferation of pathogens in the vast diversity of bases where they are active. When pathogenic bacteria are introduced into the growing Environment, they colonize the plants and remain attached to fresh produce through several mechanisms.

3.2 Contamination prevention

To guarantee production protection on a sustainable scale, it is imperative to properly recognize the paths of pathogen's entry, fortune, carriage, establishment, and survival in the agricultural Environment like soil, irrigation water, manure, seeds, and other tools and sources. Rhizosphere microbiota has been widely and deeply studied for decades; however, leaf microbiota and biofilms in irrigation systems are still ineffectively explored. The sources of microbiota on plant leaves have not been fully known until today. Phyllosphere is diverse in structure, and microorganisms can enter via air, soil, plants, animals, insects, birds, water, and other circumstantial materials (Vorholt, 2012)

4. Plastic use and challenges

4.1 Plastic a need of everyday life

Plastic use is inevitable in daily life and can be noticed on beaches, streets, gutters, storm drains, wedged in trees, behind bushes, and stomachs of dead animals in massive quantities (Pacific, 2019; Rodrigues et al., 2019). Plastics are ubiquitous, and investigators are just beginning to know how to handle the adverse effects of rising plastic pollutants on the ecosystem (Forrest et al., 2019). The range of plastic contamination and its impacts on the biosphere is alarming and needs massive strategies to deal with the challenges (Almroth and Eggert, 2019; Gross and Enck, 2021). The contributors to plastic pollution are significant in numbers and unavoidable. Plastics can potentially interfere with the life activities of humans, animals, forests, oceans, and agriculture, highlighting the emergency for developing vital tools and approaches to solve the problem of plastic pollution (Chae and An, 2018; Vriend et al., 2021).

4.2 Plastic production

Plastic products are a matter of routine use worldwide, and their kinds and amounts of utilization differ by area and region, which depends on mechanization and supply chain. In 2021, 390.7 million tonnes (Mt) of plastic

were produced globally. 90.2% of the 390.7 million tonnes of plastic is fossil-based, 8.3% post-consumer recycled, and 1.5% bio-based/bio-attributed (Fig. 1). In North America (18%), EU27+3 (15%), China (32%), Japan (3%), Latin America (4%), Middle East Africa (8%), the rest of Asia (17%) and CIS[2] (3%) of 390.7 million tonnes plastic were produced (Fig. 2). 4% of this global plastic was used in farming, gardening, and agriculture in 2021.

5. Plastic application in agricultural

The plastic product application broadly involves protecting, distributing, retailing, and maintaining quality in agriculture, livestock, aquaculture, and fisheries. The main types of agricultural plastic include surface mulching films, tunnels, greenhouses, nets, irrigation tubes, fleece, driplines, sacks, bottles, silage films, coatings on fertilizers, pesticides, seeds, fruit protectors, plant protectors, ropes, lines, traps, enclosures, bale wrap, plastic bags, ear tags, tree shelters, fishing gear, pesticide containers, polymer-coated controlled release fertilizers, nets used for aquacultural and fisheries operations (Fig. 3) (FAO, 2021). These ubiquitous plastics carry possible microorganisms which induce health risks to plants and produce consequences. Plastics carry pathogenic microbes and support the genetic element's horizontal exchange in the niche, which can initiate pathogen evolution in a virgin habitat (Maraveas, 2020; Serna-Abascal et al., 2022). The specific interaction mechanisms and impacts of plastic-associated microbiomes on crops remain unknown.

6. Plant-microbe interaction investigation

Understanding plant-microbe interaction includes genetic, molecular, and biochemical approaches; however, the recent past has witnessed important technical innovations in super-resolution microscopy and cryo-electron microscopy techniques to view microbes at the nanoscopic level. Moreover, new tools and algorithms for live imaging and data processing have been established, and evolving subfields in cell biology extend researchers valued viewpoints for more mechanistic studies. Overall, current advances in cell biology provide extraordinary chances to study plant-microbe interaction at the cellular, molecular and nanoscopic levels (Dal Cortivo et al., 2017). Advanced RNA., DNA., genome, proteome certification, and analysis techniques are used to discover plant-microbe interaction mechanisms for crop improvement (Douglas and Werren, 2016). If the features of microbial communities in the rhizosphere, phyllosphere, and on the surfaces of the irrigation system are beneficial and influential could be used as a sustainable alternative for crop improvement and productivity in long-term schemes (Vishwakarma et al., 2020).

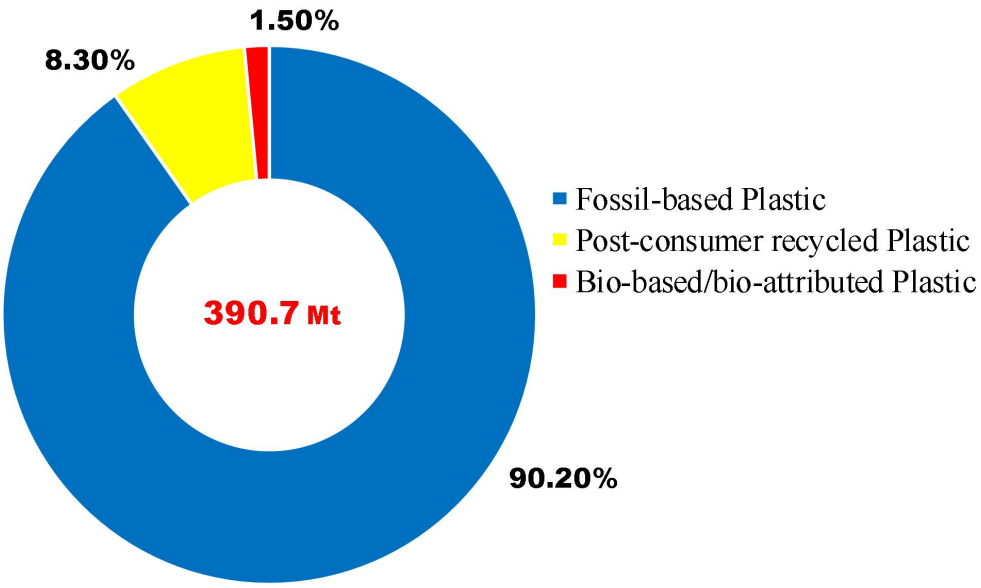

Figure 1. Global plastics production in 2021 (Data source: Plastics Europe e.V., 2021)

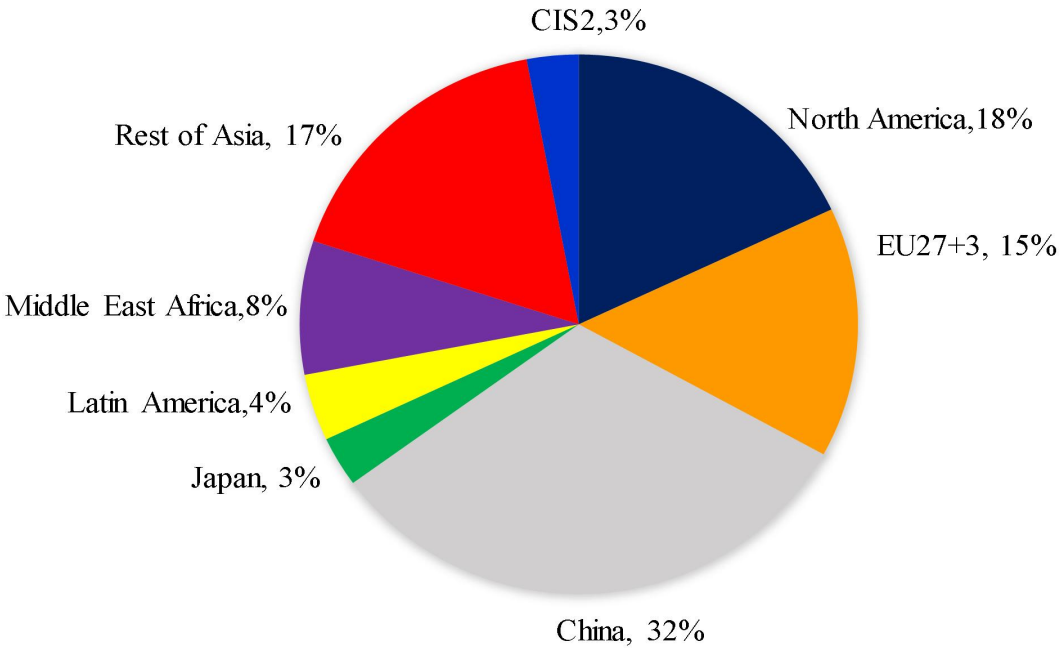

Figure 2. Worldwide distribution of plastics production in 2021 (Data source: Plastics Europe e.V., 2021).

Figure 3. Use of plastic in agriculture for different purposes. A: Mulching films, B: Bunker covers, C: Bale nets and twines, D: Ropes, E: Fishing nets, F: Hydroponic irrigation pipes, G: Plastic cane as pots (ISBN 978-92-5-135402-5. ©FAO, 2021)

7. Plant microbiomes

Plants are exposed to various communities of microorganisms, and microbiota colonizes all accessible tissues. The microbiome resides in the endosphere, phyllosphere, rhizosphere nutrient solutions, and irrigation system, creating host-plant interaction (Fig.4). Environment induces interactions and increases plant resilience to subsequent stresses (Naylor and Coleman-Derr, 2018; Trivedi et al., 2020). Promising advancements have been made in recognizing the impact of the microbiome on plant growth and development, but the traits responsible for plant resilience to environmental conditions need to be identified. The advanced research on plant-microbiome interaction is essential for forecasting and evaluating the effects of climate alteration on key competence and diversity.

7.1 Phyllosphere microorganisms

The phyllosphere is an ecosystem, unlike the rhizosphere and endosphere (Enespa and Chandra, 2022). The phyllosphere contains prokaryotes, eukaryotes, viruses interacting mutually, and their host plants (Lindow and Brandl, 2003). Limited nutrient availability and changing climatic circumstances make the phyllosphere an active and stressful environment for its microbiomes (Couée et al., 2006; Trouvelot et al., 2014). Future investigation into phyllosphere microbiomes and stimulants may clarify the mechanisms and operations that regulate the connection between plants and microbiomes in the atmosphere.

7.2 Endosphere microorganisms

Microorganisms called endophytes which penetrate and inhabit the internal tissues of plants, create the endosphere microbiome. The scientific community was with opinion for a long time; plants with no indications of illnesses have no microorganisms, generally bacteria (Compant et al., 2021). Endophyte is a habitat containing all those microorganisms that spend entire or part of their life in internal tissues (Hardoim et al., 2015). Endophytic microbes have drawn much attention because of their mysterious interaction with plants. Having enough knowledge about endophytic microbial life the endosphere is still taken as a

habitat with no fit conditions for microbial diversity (Theis et al., 2016). So, it is critical to investigate endophytic microbial groups, existing environmental factors, and their roles in improving plant growth and development with their impacts on yield.

7.3 Rhizosphere microorganisms

A thin area of soil around the roots of a plant which is influenced by numerous factors, such as root exudates and related soil microorganisms, is termed a rhizosphere (Chesworth, 2008). The rhizosphere is a significant portion of soil that is accountable for several metabolic processes, like the cycling of nutrients and carbon uptake (Ng and Ng, 2014; Singh, 2013). The roots of plants generate an interface between the soil environment and the plant, which hosts a massive pool of microbial communities (Hakim et al., 2021). The rhizosphere contains diverse microorganisms such as bacteria, algae, parasites, fungi, and viruses (Kumar and Dubey, 2020). Rhizosphere microbiota shield plants against phytopathogens and promotes plant growth and development by producing plant growth hormones. It also helps plants resistance against environmental unrests like irregular variations in temperature, salinity, drought, and all other harshnesses (Burghardt, 2020; Lu et al., 2018). However, discovering the mechanism present under the interaction of plants and rhizosphere microorganisms still needs further investigation.

8. Advanced probing tools and techniques

Currently many microbial investigating tools and techniques are available including microbial cultivation by plating methods, electron microscopy-SEM-TEM (Goldsmith and Miller, 2009; Wisse et al., 2010) , nucleic acid extraction, DNA sequencing, PCR/qPCR (Del Mar Lleò et al., 2000; Garibyan and Avashia, 2013; Khan et al., 2016; Overbergh et al., 2003; Pagano et al., 2011; v. Wintzingerode et al., 1997), clone genomic libraries, stable isotope probing, microarrays, next generation technique (NGS) metagenomics (Marchev et al., 2021), transcriptomic (Handelsman, 2004; Khan et al., 2021), proteomics (Khan et al., 2022), molecular finger printings techniques, denaturing gradient gel electrophoresis (DGGE) (Al‐Mailem et al., 2017), terminal-restriction length polymorphism (T-RFLP) (Portillo et al., 2011), temperature gradient gel electrophoresis (TGGE) (Ritchie et al., 2000), single-strand conformational polymorphisms (SSCP) (Johnston‐Monje and Lopez Mejia, 2020), ribosomal internal spacer analysis (RISA) (Osborn et al., 2000) , length heterogeneity-PCR (LH-PCR) (DeAngelis et al., 2011), random amplified polymorphic DNA (RAPD) (Bardakci, 2001; Broadway, 2012) and biosensors (Gavrilaș et al., 2022) etc. However, the methods, tools, and techniques are selected according to the need of the research goals and directions.

9. Conclusion

The rising global population at an alarming

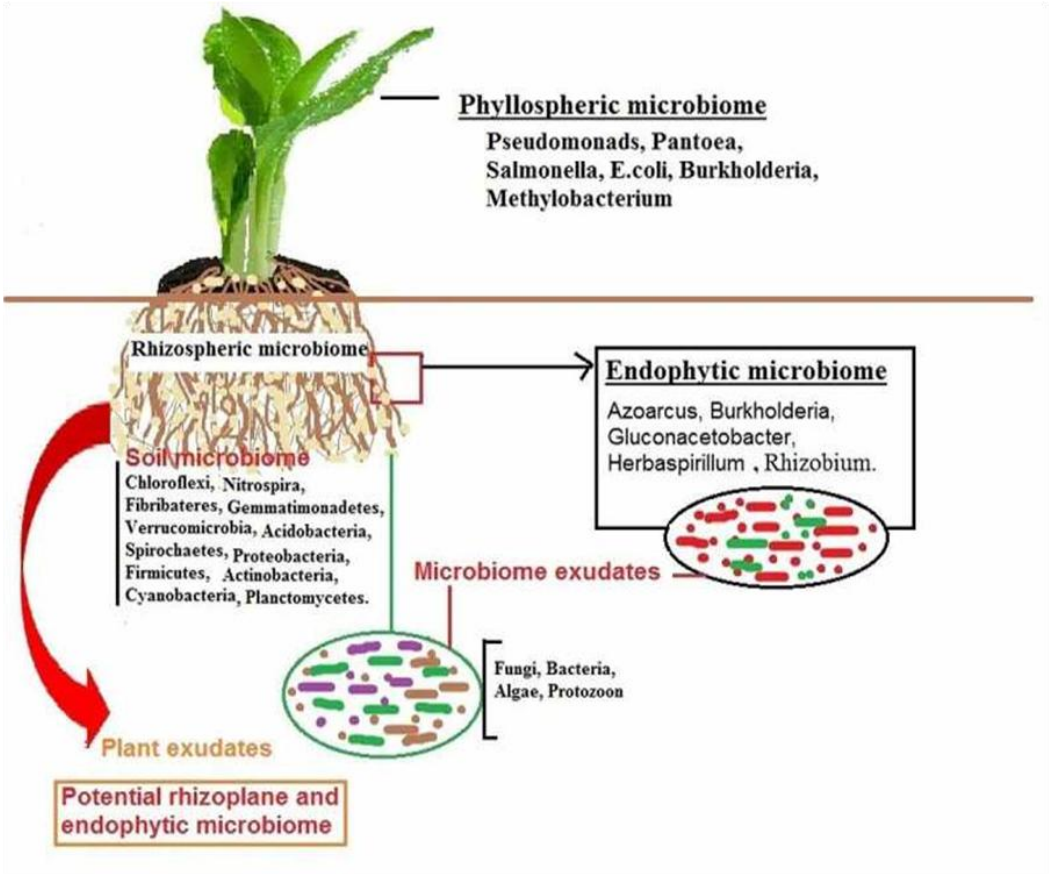

Figure. 4. Plant microbiomes classification (Enespa and Prem Chandra, 2022)

rate requires enhanced crop production to sustain the world's food needs supply and ensure agricultural sustainability. Missing feasible replacements for plastic use in agricultural activities made it impractical to avoid their utilization, and there are no silver bullets to eradicate the challenges due to plastic application. Plastic use in agricultural activities may have worthy advantages in the short term; however, long-term negative impacts cannot be overlooked, particularly as microbes host. Detection of microbiomes in rhizospheric, phyllospheric, and endophytic zones of plants and the mechanism of their interactions are highly important. A wide range of advanced tools and techniques is available which could be applied to achieve comprehensive knowledge about microbes' diversity, interaction mechanism, and positive or negative impacts on plants, especially crops. Understanding the microbe's diversity, lethality, and positive roles in a particular habitat will enable scientists to design precautionary measures against contamination and subsequent losses. The isolation and identification of beneficial microbial strains could be used in crops to achieve commercial targets. A comprehensive understanding of the underlying mechanism in plant microbes' interaction is necessary, which could be reached via novel methodologies and protocol optimization. The development of precautions and principles applied in sampling, packing, preservation, storage, transportation, and

disposal to minimize the risk of contamination is also a substantial need.

10. Future perspectives

From the commercial point of view, it would be valuable to recognize the strains and their roles in a crop cultivated in a particular environment rather than detecting whole microbiota. The investigations should focus on the microbes in nutrient solutions, irrigation systems, all agricultural use plastics, and plant parts above and below ground. Knowledge of molecular mechanisms underlying plant-microbe interaction will be useful for manipulating and developing strategies in the future. Disclosing microbial effects on resource use efficiency in the crop will facilitate precise cost-effective analysis. Identifying novel plant growth-promoting microbes will reduce agricultural costs and environmental pollution. Genetic and chemical analysis of exudates is essential to understand microbe's recruitment in the rhizosphere. Comprehensive elucidation of plastic microbiomes' nature and their interaction with plants is crucial to better manage the damaging risks in the agricultural economy. Identified beneficial microbes could be used to improve plant growth and production. It will provide the foundation for the enhanced governing system with broad plastic application in agricultural practices locally and globally.

Authors Contributions

Q.K designed the research, wrote the manuscript, and contributed to all aspects of the paper. M.K contributed to the microbial section. S.J.S added to the plastic section. The Author (s) read and approved the final manuscript.

Availability of Data and Materials

Not Applicable.

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ORIGINAL RESEARCH

Evaluation of integrated nutrient management on soil health, maize productivity and grain quality

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ABSTRACT: Managing various organic residues produced from agricultural waste is today's prominent need. The present experiment was conducted to evaluate the effect of integrated, chemical, and organic fertilizers on maize productivity. Initially, overoptimistic was prepared using different organic residues *viz.*, paddy straw, neem leaves and dhaincha leaves, each in combination with cow dung in 1:1 ratio. Further, prepared overoptimistic along with integrated nutrient and chemical fertilizer treatments, were tested on maize productivity. The experiment was carried out in Randomized Block Design. The average two-year data revealed the increased yield and yield attributes of maize with integrated nutrient management followed by the recommended dose of fertilizers and different overoptimistic treatments. The least maize productivity was noted with control treatment. The different overoptimistic treatments comparatively improved the organic carbon (0.43 to 0.45%) and micronutrient status of the soil in second year of application (Fe- 10.85 to 13.32 mg kg^{-1}, Zn- 2.95 to 4.18 mg kg^{-1}, Cu- 0.55 to 0.73 mg kg^{-1}, Mn- 10.37 to 15.24 mg kg^{-1}). The result of overoptimistic application can be recorded higher in terms of improvement in yield and soil properties in the later years, as the initial organic carbon and nutrient content of the experimental soil was recorded to be low, and, it takes almost three to four years for positive response of soil to the applied organic amendments. Therefore, long-term experiments are required to evaluate the effects of overoptimistic on soil chemical properties and maize productivity. The investigation revealed that integrated nutrient treatment proved better in terms of improving the yield and nutrient status of the soil.

KEYWORDS: Cob, earthworms, grains, soil; mineralization

1. Introduction

Maize is the most significant crops in India and all over the world (Sadiq et al., 2017). The history of maize regarding its cultivation was in Europe, Oceania, Asia, and Africa, isn't much ancient, despite the fact that the climatic conditions in these regions are suited for its production (Majnoon 2006; Bolanle et al., 2022). Recently, the cultivation and consumption of maize crop in different regions have risen substantially around the world, possibly due to its relevance in the production of various products. Maize requires several types of sustenance throughout its growth that can be supplied from chemical fertilizers. Among different elements nitrogen is among the most significant components utilized in maize production, and a lack of it might limit the

nutritional elements of this crop (Wu et al., 2021; Khalid and Islam 2001).

Global hunger levels remain disturbingly high. According to the findings of the GRFC 2022, they eclipsed all prior records reported by the Global Report on Food Crises (GRFC) in 2021, with nearly 193 million people severely food deprived and in dire need of immediate assistance throughout 53 countries/territories. This marks an almost 40 million people increase over the previous high set in 2020 (GRFC 2021) (2022 Global Report on Food Crises/fao.org). Traditional farming no longer overcome the food demand; while chemical fertilizers have led to high production, they have also aggravated environmental damage. Effective management and application of balanced inputs is required for a fruitful establishment of crops (Dai and Dong 2014, Amin et al. 2017), particularly nitrogen, phosphorus, and potassium being critical elements for both cereal legume inter-crops (Divitoand Sadras 2014; Ali et al., 2019). In modern agriculture, the overuse of chemical fertilizers for high agricultural output has become popular (Ali et al., 2020). As a result, chemical fertilizers by itself are unable to sustain long-term fertility; thus, these fertilizers can be combined with overoptimistic, which is generated by the combination of various waste organic sources. This is known as co-composting, and it has been established profitable by increased wheat crop yields using animal manure, sludge leftovers, sawdust, and wood chips. This application of chemical fertilizers along with organic manure is growing popular among farmers due to growing awareness and enhanced productivity while maintaining soil fertility. Another study found that using organic fertilizer instead of chemical fertilizer raised soil pH and nutrient content while increasing microbial biomass (Iqbal et al., 2020). As a result, researchers recommended the use of organic compounds for increased crop yield and nutrient-rich soil preservation over a prolonged period of fertility conditions. Along with increased production, these fertilizers and pesticides have a deleterious influence on human health and reduce soil nutrient productivity and efficiency. Due to the environmental degradation caused by excessive nitrogen fertilizer use, an alternate technique based on biological origin, appropriate, and less expensively manufactured for nitrogen management is required. The use of overoptimistic to substitute nitrogen fertilizer in the soil can help to reduce environmental pollution created by removing nitrate from the soil (Raza et al., 2022). Additionally, the use of overoptimistic along with manure resulted in a better and significant enhancement in the functioning of seeds of maize and oats (Nanjappa et al. 2001; Jayanthi et al. 2002). According to research findings, using overoptimistic not only improves plant growth but also improves crop performance (Raza et al., 2022). The application of overoptimistic in sustainable agriculture increased the beneficial soil microorganisms, including mycorrhizal fungi and phosphate-dissolving bacteria and fungi.

Keeping these considerations in view the present study entitled "Evaluation of different organic waste materials for preparing overoptimistic and their effect on maize productivity" was conducted with the objective to study the effect of different rates

of prepared overoptimistic on growth and yield of maize.

2. Material and methods

2.1 Experimental deign and location

The present field experiment was conducted during Kharif 2019-20 and 2020-21 at the Integrated Farming System Farm, Punjab Agricultural University, Ludhiana situated at 30°54'N latitude and 75°48'E longitude at an altitude of 247 m above sea level. Initially, overoptimistic was prepared using different organic residues such as paddy straw (*Oryza sativa*), neem leaves (*Azadirachta indica*), dhaincha leaves (*Sesbania bispinosa*) and were combined with cow dung in 1:1 proportion i.e. {Paddy Straw + Cow dung (1:1)}, {Neem leaves + Cow dung (1:1)}, {Dhaincha leaves + Cow dung (1:1)} and {Cow Dung (100%)} for preparation of overoptimistic. The prepared overoptimistic (sieved through 4mm sieve) from different residues were tested on maize crop. The maize variety PMH 1 was sown in last week of July in 2019-20 and first week of July in 2020-21 (Kharif season) with recommended package of practices of PAU.The experiment includes seven treatments with three replications and was carried out in Randomized block design. The different treatments were control, RDF (Recommended dose of fertilizers), RDF+FYM (Recommended dose of fertilizers + Farmyard manure), Paddy straw + cow dung (1:1) + 1kg earthworms, Neem leaves + cow dung (1:1) + 1kg earthworms, Dhaincha leaves + cow dung (1:1) + 1kg earthworms. The harvesting was done in the first week of November 2020 during first year and last week of October 2021 in the second year.

2.2 Soil Analysis

The experimental soil was of loamy sand texture. Before the start and end of experiment, soil samples were collected and analyzed for various chemical properties. The initial sample of soil used for cultivation of maize crop was with neutral pH (7.2), normal electrical conductivity (EC) (0.37 dS m^{-1}) and low organic carbon (OC) content (0.38 %). The soil was recorded with low available nitrogen (AN) (109.8 kg ha^{-1}), medium available phosphorus (AP) (16 kg ha^{-1}) and medium available potassium (AK) (168.0 kg ha^{-1}). The soil pH and EC was analyzed with 1:2 soil:water suspension (Jackson 1967). The organic carbon (OC) percent of soil was analyzed with Walkley and Black's rapid titration method (Walkley and Black 1934). The macronutrient content *viz,* nitrogen, phosphorus and potassium (N, P & K) content of soil was analyzed with modified alkaline potassium permanganate method (Subbiah and Asija 1965), 0.5N Sodium bicarbonate extractable P by Olsen's method (Olsen et al. 1954) and ammonium acetate extractable K method (Jackson 1967) respectively. Whereas, the soil micro-nutrient content was analyzed by atomic absorption spectrophotometer method (Lindsey and Norvell 1978).

2.3 Grain and straw analysis

The straw and grain samples were also collected after harvesting for analysis of N, P, K and micro-nutrient content. The total nitrogen (TN) content was analyzed by Kjeldahl digestion procedure as given by Bremner and Hauck (1982), total phosphorus (TP) content measured using the Vanado-Molybdo-Phosphoric yellow colour method (Jackson 1967) and total potassium (TK)

content was analyzedby Flame Photometer (Jackson 1967). The micronutrient content (Fe, Cu, Zn, Mn) of maize grains and straw was analyzed by atomic absorption spectrophotometer (Lindsay and Norvell 1978).

2.4 Growth and yield attributes

Plant height, ear length, Number of grains per row, tassel size, grain size, 1000 grains weight, maturity days and yield were noted. The data was recorded before harvesting of crop.

2.4.1 Plant Height

Ten plants from each plot was randomly picked and plant height was measured. The mean values were recorded from each plot in cm. The measurement of plant height was done before harvesting from ground level to the whorl base.

2.4.2 Number of leaves per plant

The leaves number per plant were counted ignoring the dry leaves at the base of plant from ten randomly selected plants and the mean values was recorded.

2.4.3 Ear Length

Ten ears per plot was randomly selected and ear length was measured. The ear length was measured from base to tip of the ear and the mean values were noted from each plot in cm.

2.4.4 Number of grains per row

The grains number per row was recorded from five randomly selected cobs per plot were counted and mean values were recorded.

2.4.5 Tassel size

Ten tassels per plot was randomly selected and tassel size was measured from base to the tip. The mean values were recorded from each plot.

2.4.6 1000 grain weight

Thousand grains were taken and then counted manually from each plot and weighed in grams.

2.4.7 Cob length (cm)

Five cobs per plot were randomly selected and cob length was measured. The mean values were recorded from each plot in cm.

2.4.8 Number of rows per cob

The Number of grain rows from five representative cobs selected randomly from each plot were counted and mean values recorded.

2.4.9 Number of grains per cob

No. of grains per cob were worked out by multiplying no. of rows by no. of grains per row from five cobs randomly selected from each plot.

2.4.10 Grain size

The thickness and length of the grains was recorded in mm with vernier caliper. The size of randomly selected five grains was measured and the average was recorded as grain size from each plot.

2.4.11 Grain yield

All the cobs from net plot were left for sun drying for about fifteen days and threshing was carried out. The grain yield was adjusted to 15 per cent moisture level and expressed as q ha^{-1}.

2.4.12 Stover yield

After picking the cobs, the leftover plant material, including the husk, was left for sun drying, weighed, and presented as stover yield (q ha^{-1}).

2.5 Statistical analysis

Statistical analysis of the different parameters were analyzed with the help of analysis of variance (ANOVA) technique (Gomez and Gomez 1984) for randomized block design using CPCS1 software developed by the Department of Mathematics and Statistics, PAU, Ludhiana (Cheema and Singh 1991). The data were compared with a significance level test at $p< 0.05$.

3. Result and Discussions

3.1 Soil properties

3.1.1 pH, EC and OC

Soil pH in the most important factor, which affect nutrient availability in soil (Brandy and Weil 1996; Devkota et al., 2022; Ali et al., 2022). In the present study, the initial soil was 7.2. In this two-year experiment, no significant differences were noted among different treatments and the pH lies in the neutral range (Table 1). EC of soil is the measurement of amount of salts in the soil (salinity). It is a good predictor of loss and availability of nutrients, soil texture, and accessible water capacity. Compared to the initial soil sample, the slight increase was recorded as presented in table 1. On an average, the higher soil EC was recorded with Dhaincha leaves overoptimistic treatment which lies at par to other overoptimistic treatments but showed slight increase in comparison to integrated nutrient treatment, RDF and control treatments. With the increase in soil organic carbon levels, it leads to improved soil health and hence crop yield. The average of two years data represented a comparatively slight increase in the soil organic carbon content with different overoptimistic treatments (Table 1). The soil supplied with neem leaves overoptimistic and dhaincha leaves overoptimistic showed significant increase in comparison to the control treatment. This might be due to the fact that overoptimistic upon decomposition improves the organic carbon status of soil. Chimdessa and Sori (2020) reported that soil organic carbon status was higher with the overoptimistic (1.5 and 3 tons ha^{-1}) and integrated nutrient treatment (i.e. overoptimistic (3 ton ha^{-1} + NPS fertilizers 200 kg ha^{-1}) than chemical fertilizer treatment. Nasrin et al. (2019) revealed that the soil organic carbon content increases with the overoptimistic at 12 tons ha^{-1} in comparison to control treatment.

3.1.2 Available N, P, K and micronutrient content

The roles of nitrogen, phosphorus and potassium as the important macronutrients that are essential for regular growth and development of crop plants (Uchida 2000). The various treatments as followed in present experiment expressed no considerable differences in the soil available nitrogen and phosphorus content whereas, average data revealed the significant variations in the soil available potassium content among different treatments (Figure 1 and 2). The increased K content was observed with integrated nutrient management treatment (174.67 kg ha^{-1}) which lies at par to the neem leaves overoptimistic (174.28 kg ha^{-1}) and dhaincha leaves overoptimistic (173.39 kg ha^{-1}) treatments but showed significant increase of 2.36% and 2.43% in comparison to paddy straw overoptimistic and cow dung overoptimistic treatments respectively. Priyanka et al. (2019) investigated that the reason for increased soil available K content with integrated nutrient management may be because, FYM is noted

as best K source as well as retain the K^+ ions on exchangeable sites by decreasing its loss through leaching and organic matter and clay interaction responsible for K release.

Micronutrients are the elements required by the plants in very small amounts. Considerable differences were noted in the micronutrient content of the soil (Figure 1-2). The present study conducted has shown the increased available micronutrient content with different overoptimistic treatments in comparison to INM and control treatments. The higher soil available Fe content was recorded with paddy straw overoptimistic treatment and higher Zn, Cu and Mn content with dhaincha leaves overoptimistic treatment. This might be due to the higher micronutrient content of overoptimistic which are otherwise not supplied by the synthetic NPK fertilizers.

3.2 Maturity days

The differences noted in the maturity of maize crop among different treatments have shown very slight variations. The crop supplied with integrated nutrient management and RDF treatments was matured comparatively earlier such as 98 days after sowing in comparison to different overoptimistic treatments such as 102 days after sowing. This might be due to the readily and timely availability of nutrients due to increased mineralization rate in the integrated nutrient management and RDF treatments and hence dry matter accumulation rate is faster and vegetative growth period is reduced in comparison to the overoptimistic treatments. Vermicompost being slow-release fertilizer, supply nutrients slowly and steadily depending on its mineralization rate and hence extend the vegetative phase of the crop, therefore, delaying the maturity of crop.

3.3 Yield attributing characteristics of maize

3.3.1 Plant height

Plant height is an index of growth and development that represents the infrastructure build-up over time, and also an indicator of growth promoting and suppressing ability of treatments. In the current study, plant height was significantly influenced with the overoptimistic and chemical fertilizer treatments (Table 2). During first and second year the maximum plant height was obtained in integrated nutrient management (INM) treatment (208.6 cm) which is in close count of RDF treatment (208.0 cm) (Table 2). INM treatment as followed by different overoptimistic treatments which further showed significant difference in comparison to the control treatment. The conclusion could be attributed to the fact that the regular and timely supply of nitrogen with integrated nutrient management and RDF treatments is responsible for comparatively increased plant development during initial growth stages because nitrogen improves the vegetative growth, which further responsible for higher production of photosynthetic materials and hence improve the plant height, while organic fertilizers improved plant growth during later growth stages. Prajapati et al. (2018) reported increased plant height with integrated nutrient treatment (100 per cent overoptimistic + 100 percent RDF) in comparison to control, RDF and overoptimistic treatments.

Table 1: Effects of inorganic fertilizer application on pH, EC & OC content of soil after harvesting of maize.

T	pH (1:2)			EC (dS m^{-1})			OC (%)		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	7.20 ± 0.06	7.40 ± 0.12	7.30 ± 0.03	0.38 ± 0.01	0.43 ± 0.01	0.40 ± 0.00	0.38 ± 0.01	0.39 ± 0.01	0.38 ± 0.00
T_2	7.34 ± 0.05	7.47 ± 0.13	7.40 ± 0.04	0.39 ± 0.01	0.47 ± 0.01	0.43 ± 0.00	0.40 ± 0.01	0.41 ± 0.02	0.40 ± 0.01
T_3	7.21 ± 0.06	7.37 ± 0.15	7.29 ± 0.04	0.41 ± 0.01	0.48 ± 0.01	0.44 ± 0.00	0.41 ± 0.01	0.43 ± 0.01	0.42 ± 0.00
T_4	7.20 ± 0.06	7.32 ± 0.13	7.26 ± 0.04	0.42 ± 0.01	0.49 ± 0.01	0.45 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.41 ± 0.00
T_5	7.15 ± 0.07	7.24 ± 0.12	7.19 ± 0.09	0.44 ± 0.01	0.53 ± 0.02	0.48 ± 0.01	0.42 ± 0.01	0.44 ± 0.02	0.43 ± 0.01
T_6	7.06 ± 0.01	7.18 ± 0.07	7.12 ± 0.04	0.45 ± 0.01	0.56 ± 0.01	0.50 ± 0.01	0.43 ± 0.01	0.45 ± 0.02	0.44 ± 0.01
T_7	7.16 ± 0.07	7.28 ± 0.14	7.22 ± 0.11	0.43 ± 0.01	0.51 ± 0.01	0.47 ± 0.01	0.40 ± 0.01	0.42 ± 0.01	0.41 ± 0.01
CD (p=0.05)	ns	ns	ns	0.03	0.04	0.02	ns	ns	0.03

Table 2: Effects of inorganic fertilizer application on maize crop yield attributing characteristics.

T	Plant height (cm)			Tassel size (cm)			Ear length (cm)			No. of leaves		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	162.8 ± 1.8	164.7 ± 1.1	163.8 ± 0.8	24.0 ± 0.2	24.3 ± 0.3	24.2 ± 0.2	21.5 ± 1.0	21.8 ± 0.9	21.6 ± 1.0	11.0 ± 0.3	11.2 ± 0.3	11.1 ± 0.3
T_2	208.0 ± 1.7	210.5 ± 1.0	209.3 ± 1.2	27.0 ± 1.4	27.4 ± 1.4	27.2 ± 1.4	26.6 ± 2.0	27.0 ± 2.1	26.8 ± 2.0	12.4 ± 0.3	12.6 ± 0.4	12.5 ± 0.3
T_3	208.6 ± 1.7	211.9 ± 1.2	210.2 ± 0.7	28.9 ± 0.6	29.1 ± 0.4	29.0 ± 0.5	28.8 ± 0.2	29.2 ± 0.3	29.0 ± 0.2	12.4 ± 0.3	12.7 ± 0.2	12.6 ± 0.3
T_4	175.7 ± 1.5	179.5 ± 1.3	177.6 ± 1.4	26.7 ± 1.4	27.1 ± 1.3	27.0 ± 1.4	25.5 ± 0.9	26.1 ± 0.8	25.8 ± 0.8	11.0 ± 0.1	11.3 ± 0.1	11.1 ± 0.1
T_5	195.7 ± 1.7	199.7 ± 1.2	197.7 ± 1.5	27.2 ± 1.0	27.4 ± 0.9	27.3 ± 0.9	26.0 ± 0.9	26.3 ± 0.9	26.1 ± 0.9	11.1 ± 0.3	11.3 ± 0.3	11.2 ± 0.3
T_6	183.9 ± 1.7	189.6 ± 1.1	186.7 ± 0.8	27.1 ± 1.6	27.3 ± 1.6	27.2 ± 1.6	25.9 ± 1.3	26.2 ± 1.4	26.0 ± 1.3	11.1 ± 0.4	11.4 ± 0.4	11.2 ± 0.4
T_7	174.3 ± 1.7	181.2 ± 1.2	177.8 ± 0.8	25.8 ± 1.6	26.0 ± 1.5	26.0 ± 1.5	25.6 ± 1.7	25.8 ± 1.7	25.7 ± 1.7	11.0 ± 0.2	11.3 ± 0.3	11.2 ± 0.2
CD (p=0.05)	7.4	3.4	3.9	ns	ns	2.2	ns	ns	2.6	0.9	0.9	0.6

T_1- Control, T_2- RDF (Recommended dose of fertilizers), T_3- RDF+FYM (Recommended dose of fertilizers + Farmyard manure), T_4- {Paddy Straw + Cow dung (1:1)} + 1kg earthworms, T_5- {Neem leaves + Cow dung (1:1)} + 1kg earthworms, T_6- {Dhaincha leaves + Cow dung (1:1)} + 1kg earthworms and T_7- {Cow Dung (100%)} + 1 kg earthworms.

Table 3: Effect of inorganic fertilizer application on yield attributing characteristics of maize cobs.

T	Cob length (cm)			Cob weight (g)			Grains per cob			Grain rows		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	13.6 ± 0.4	14.9 ± 0.2	14.3 ± 0.3	104.2 ± 1.4	119.5 ± 1.9	111.8 ± 0.6	374.9 ± 1.8	385.5 ± 1.3	380.2 ± 1.6	13.4 ± 0.2	13.6 ± 0.2	13.4 ± 0.2
T_2	18.3 ± 0.7	20.6 ± 0.5	19.5 ± 0.6	171.8 ± 1.9	191.5 ± 2.4	181.7 ± 1.8	463.1 ± 1.6	453.1 ± 2.2	444.6 ± 1.2	13.8 ± 0.1	14.0 ± 0.1	13.8 ± 0.1
T_3	18.8 ± 0.1	21.5 ± 0.4	20.2 ± 0.2	175.0 ± 1.3	196.0 ± 2.2	185.5 ± 1.6	459.4 ± 1.6	480.4 ± 1.9	469.9 ± 0.2	14.0 ± 0.1	14.2 ± 0.1	14.0 ± 0.1
T_4	15.9 ± 0.2	18.6 ± 0.3	17.2 ± 0.2	132.6 ± 1.8	162.6 ± 1.6	147.6 ± 0.9	411.2 ± 1.1	425.9 ± 2.1	418.5 ± 1.6	13.4 ± 0.1	13.6 ± 0.1	13.6 ± 0.1
T_5	16.5 ± 1.3	19.4 ± 0.8	18.0 ± 1.1	167.6 ± 2.3	189.0 ± 2.1	178.3 ± 1.1	432.6 ± 1.8	455.7 ± 2.0	444.2 ± 0.9	13.8 ± 0.2	14.0 ± 0.2	14.0 ± 0.2
T_6	15.8 ± 0.2	19.1 ± 0.5	17.5 ± 0.2	154.2 ± 2.3	183.2 ± 1.9	168.7 ± 1.5	428.6 ± 1.8	449.7 ± 1.7	439.2 ± 0.7	13.8 ± 0.1	13.8 ± 0.1	13.8 ± 0.1
T_7	15.8 ± 0.5	18.9 ± 0.6	17.4 ± 0.6	132.9 ± 1.6	168.6 ± 2.5	150.8 ± 1.3	422.3 ± 2.0	437.4 ± 1.2	429.8 ± 1.1	13.6 ±0.2	13.8 ± 0.1	13.8 ± 0.1
CD (p=0.05)	1.9	1.6	1.2	5.9	6.8	4.3	5.6	5.5	3.7	ns	ns	ns

Note: For treatments detail see table 1.

Table 4: Effect of inorganic fertilizer application on yield attributing characteristics of maize cobs.

Treatment	**Grains/row**			**Grain size**						**1000 grain weight (g)**		
				Grain length(mm)			**Grain thickness(mm)**					
	2019-20	**2020-21**	**Mean**	**2019-20**	**2020-21**	**Mean**	**2019-20**	**2020-21**	**Mean**	**2019-20**	**2020-21**	**Mean**
T_1	22.7 ± 0.4	28.1 ± 0.5	27.9 ± 0.5	8.9 ± 0.4	8.9 ± 0.5	8.9 ± 0.4	4.8 ± 0.1	5.0 ± 0.3	4.9 ± 0.1	200.6 ± 2.4	204.2 ± 2.6	202.4 ± 2.5
T_2	32.5 ± 0.2	32.8 ± 0.1	32.7 ± 0.1	9.2 ± 0.7	9.3 ± 0.7	9.3 ± 0.7	4.9 ± 0.1	5.0 ± 0.2	5.0 ± 0.1	301.8 ± 3.9	305.1 ± 3.6	303.4 ± 3.8
T_3	32.7 ± 1.0	33.0 ± 0.9	32.8 ± 0.9	10.1 ± 0.1	10.1 ± 0.1	10.1 ± 0.1	5.1 ± 0.3	5.9 ± 0.9	5.5 ± 0.4	304.2 ± 2.6	308.5 ± 2.9	306.3 ± 2.7
T_4	27.9 ± 0.8	28.2 ± 0.7	28.1 ± 0.7	9.0 ± 0.3	9.0 ± 0.3	9.0 ± 0.3	4.9 ± 0.1	5.0 ± 0.2	4.9 ± 0.2	239.0 ± 1.2	246.3 ± 1.4	242.6 ± 1.3
T_5	30.0 ± 0.9	30.7 ± 0.8	30.3 ± 0.8	9.2 ± 0.5	9.3 ± 0.5	9.3 ± 0.5	4.9 ± 0.3	4.8 ± 0.1	4.9 ± 0.2	255.1 ± 2.6	264.4 ± 2.9	259.8 ± 2.7
T_6	28.0 ± 1.9	28.5 ± 1.8	28.2 ± 1.9	9.1 ±0.4	9.1 ± 0.3	9.1 ± 0.3	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	249.9 ± 2.0	257.5 ± 1.7	253.7 ± 1.8
T_7	28.0 ± 1.3	28.4 ± 1.0	28.2 ± 1.1	9.0 ± 0.1	9.1 ± 0.4	9.0 ± 0.2	4.9± 0.2	4.9 ± 0.1	4.9 ± 0.2	244.2 ± 1.7	250.1 ± 2.3	247.1 ± 2.0
CD (p=0.05)	3.3	2.9	2.1	ns	ns	ns	ns	ns	ns	8.2	8.4	5.6

T_1- Control, T_2- RDF (Recommended dose of fertilizers), T_3- RDF+FYM (Recommended dose of fertilizers + Farmyard manure), T_4- {Paddy Straw + Cow dung (1:1)} + 1kg earthworms, T_5- {Neem leaves + Cow dung (1:1)} + 1kg earthworms, T_6- {Dhaincha leaves + Cow dung (1:1)} + 1 kg earthworms and T_7- {Cow Dung (100%)} + 1 kg earthworms.

Preetham et al. (2020) reported the maximum plant height within integrated nutrient management treatment plots (25 per cent N through overoptimistic + 75 per cent RDF + *Azospirillum* and *Bacillus megaterium* at 5 kg ha^{-1} each) in comparison to control and RDF treatments.

3.32 Leaf number per plant, tassel size and ear length

The effect of inorganic fertilizers and different overoptimistic treatments on Number of leaves was recorded significant. The average two-year study revealed a greater number of leaves with INM treatment which lies at par to the RDF treatment (Table 2). No significant differences were observed among different overoptimistic and control treatments. The comparatively increased output of INM and RDF treatments was might be because of the increased and timely nitrogenous supply that leads to increased biochemical activity of photosynthesis which results in increased leaf number and hence dry matter accumulation. Tassel is the male flower present at the top of the corn plant. It is responsible for producing pollens and pollinates silk (female flowers) through anemophily. The average of tassel size, resulting from two-year data, lies in the range of 24.2 and 29.0 cm (Table 4). The recorded maximum tassel size was in INM treatment and minimum was in control treatment. The effect of different treatments on ear length was non-significant (Table 4).

3.4 Yield and yield components of maize

3.4.1 Cob length and cob weight

The cob length is associated to grains number in each row, which determines total Number of grains per cob and hence grain yield. The cob length varied from 13.6 to 18.8 cm during first year and 14.9 to 21.5 cm during second year whereas the cob weight lies in the range of 104.2 g to 175.0 g during the first year and increased from 119.5 to 196.0 g during second year among different treatments (Table 3). During both the years, the highest cob length and cob weight was noted in integrated nutrient management treatment, may be due to the improved and more regular supply of nitrogen and phosphorus for plant use, as nitrogen is the most important element to improve the cob size and grain numbers. Whereas, following integrated nutrient management, different overoptimistic treatments showed significant differences than control. Vermicompost, being slow-release organic fertilizer improves cob weight in comparison to control because of the more availability of nutrients due to slow and steady decomposition of microbial rich overoptimistic. Further, differences in the nutrient content of the different prepared overoptimistic (depending on the raw material used) influence the crop growth factors accordingly. The cob weight lies in the range of 104.2 g to 175.0 g during the first year and increased from 119.5 to 196.0 g during second year. Similar findings were recorded by Biswasi et al. (2020) and Preetham et al. (2020). However, during the second year, the differences in cob length between integrated nutrient management and different overoptimistic treatments were comparatively lower than that recorded in the first year.

3.4.2 Grains per cob, grain rows, grains per row and grain size

The grains number per cob has a direct impact on maize grain production. The effect of integrated nutrient management treatment was noted significant for grains per cob and grains per row in comparison to the other treatments (Table 3 & 4). The joint action of inorganic and organic fertilizer considered responsible for increasing the vegetative growth, production of photosynthetic products, flowering duration and fertility, and hence the grain number per cob. Further the effect of different overoptimistic treatments on yield attributing characteristics of maize was significant than control. Furthermore, no significant difference was observed in grain rows and grain size among different treatments (Table 3&4).

3.4.3 Thousand grains weight

Thousand grains weight (g) of maize hybrid as affected by the different treatments lies in the range of 200.6 and 304.2 g during the first year, whereas it was comparatively increased during the second year and lies in the range of 204.2 and 308.5 g (Table 4). Thousand-grains weight observed in RDF and integrated nutrient treatment was maximum and lies close to each other during both the years. Whereas both the treatments showed a significant increase in thousand grains weight compared to overoptimistic and control treatments.

3.4.5 Grain and straw yield

Gardner et al. (1985) stated that the maize grain yield is the outcome of three yield parameters product including ear number per unit area, grain number per ear and unit grain weight. Any decrease noticed in these three components (even the other components being constant), will result in the decrease of final grain yield, and therefore, the management factor that is responsible to increase any of the one parameter will ultimately contribute to increase the resultant grain yield. It was reasonably determined from the present experiment that the maximum average grain and stover yield was recorded with integrated nutrient management treatment followed by RDF, neem leaves overoptimistic, dhaincha leaves overoptimistic, cow dung overoptimistic and paddy straw overoptimistic treatments (Table 5). Whereas, the control treatment showed minimum yield. The surge in the grain yield in the second year is attributable to the average increase of maize growth and yield parameters due to soil nutrient build-up which ultimately leads to improved crop yield.

3.5 Quantitative attributes

3.5.1 Chemical analysis of maize grain and stover

The chemical analysis of maize grains revealed no significant difference in the maize grains N, K, Fe, Zn, Cu, Mn, and protein content. In contrast, a slight increase was observed in the P content of maize grains among different treatments (Table 6&7). The chemical analysis of maize stover reported with no considerable differences in N, Cu and Mn content of maize stover among different treatments, whereas considerable variations were observed in P, K, Fe and Zn content of maize stover. Following integrated nutrient management, RDF treatment showed improved nutrient content in maize stover may be because of better root and shoot

Table 5. Effect of inorganic fertilizer application on maize grain and stover yield.

Treatments	Grain yield (q ha^{-1})			Stover yield (q ha^{-1})		
	2019-20	2020-21	Mean	2019-20	2020-21	Mean
T_1	31.1 ± 1.7	32.4 ± 1.4	31.8 ± 0.8	43.2 ± 1.9	45.9 ± 1.4	44.6 ± 1.7
T_2	52.3 ± 1.6	54.8 ± 1.6	53.5 ± 1.6	84.4 ± 1.5	87.2± 1.6	85.8 ± 1.6
T_3	56.6 ± 1.8	57.2 ± 1.2	56.9 ± 1.3	93.3 ± 1.4	97.4 ± 1.4	95.4 ± 1.4
T_4	41.7 ± 1.4	45.7 ± 1.6	43.7 ± 1.2	74.1 ± 1.9	81.6 ± 1.8	77.8 ± 1.8
T_5	43.8 ± 1.7	52.9 ± 1.6	48.4 ± 1.4	77.8 ± 1.7	87.2 ± 1.4	82.5 ± 1.5
T_6	42.7 ± 1.6	51.0 ± 1.0	46.9 ± 1.2	77.6 ± 1.9	87.0 ± 1.9	82.3 ± 1.9
T_7	42.4 ± 1.7	46.4 ± 1.6	44.3 ± 1.3	76.2 ± 1.7	82.8 ± 1.6	79.5 ± 1.6
CD (p=0.05)	5.3	4.7	3.3	5.0	3.9	3.0

Note: For treatments detail see table 1.

Table 6. Effect of inorganic fertilizer application on nutrient content of Maize grain

T	N (%)	PC (%)	P (%)	K (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})
T_1	1.49 ± 0.02	9.31 ± 0.11	0.30 ± 0.02	0.58 ± 0.01	163.40 ± 1.27	20.56 ± 1.76	10.83 ± 0.59	21.64 ± 1.24
T_2	1.53 ± 0.02	9.56 ± 0.14	0.32 ± 0.02	0.67 ± 0.04	169.16 ± 1.47	23.15 ± 1.16	13.20 ± 1.18	24.76 ± 1.27
T_3	1.54 ± 0.02	9.63 ± 0.13	0.34 ± 0.02	0.69 ± 0.11	169.46 ± 1.43	23.92 ± 1.18	13.80 ± 1.57	26.00 ± 1.16
T_4	1.50 ± 0.01	9.38 ± 0.07	0.33 ± 0.02	0.74 ± 0.01	164.36 ± 1.64	22.90 ± 1.18	13.30 ± 0.55	22.48 ± 1.12
T_5	1.52 ± 0.01	9.5 ± 0.08	0.36 ± 0.02	0.73 ± 0.06	167.46 ± 1.10	23.69 ± 1.22	14.12 ± 0.61	24.32 ± 1.11
T_6	1.55 ± 0.01	9.69 ± 0.07	0.42 ± 0.01	0.76 ± 0.05	169.47 ± 1.74	23.95 ± 1.17	14.20 ± 0.52	24.86 ± 1.11
T_7	1.51 ± 0.01	9.45 ± 0.07	0.39 ± 0.02	0.74 ± 0.01	165.33 ± 1.14	23.55 ± 1.19	13.54 ± 0.49	23.08 ± 1.15
CD (p=0.05)	ns	ns	0.06	ns	ns	ns	ns	ns

Note: For treatments detail see table 1.

Table 7: Effect of inorganic fertilizer application on nutrient content of Maize stover.

T	N	P	K	Fe	Zn	Cu	Mn
	%			(mg kg^{-1})			
T_1	0.51 ± 0.01	0.15 ± 0.01	1.21 ± 0.01	194.31 ± 0.43	10.86 ± 0.96	4.05 ± 0.01	8.43 ± 1.24
T_2	0.54 ± 0.01	0.23 ± 0.01	1.51 ± 0.01	215.64 ± 0.86	14.74 ± 0.22	4.93 ± 0.05	11.05 ± 1.28
T_3	0.55 ± 0.01	0.25 ± 0.01	1.57 ± 0.01	220.12 ± 0.23	16.54 ± 0.87	5.26 ± 0.58	11.39 ± 1.04
T_4	0.52 ± 0.01	0.17 ± 0.01	1.25 ± 0.01	214.84 ± 0.57	12.87 ± 0.51	4.42 ± 0.01	9.14 ± 1.11
T_5	0.53 ± 0.01	0.19 ± 0.01	1.38 ± 0.02	217.62 ± 0.38	13.88 ± 0.25	4.78 ± 0.21	9.64 ± 1.48
T_6	0.53 ± 0.01	0.21± 0.01	1.42 ± 0.02	219.33 ± 0.24	14.08 ± 0.13	4.85 ± 0.49	10.13 ± 1.14
T_7	0.52 ± 0.01	0.18 ± 0.01	1.29± 0.01	215.53 ± 0.21	12.92 ± 0.39	4.51 ± 0.05	9.65 ± 0.99
CD (p=0.05)	ns	0.04	0.05	1.26	1.50	ns	ns

Note: For treatments detail see table 1.

Table 8. Effect of inorganic fertilizer application on nutrient uptake of maize grain.

T	Nutrient uptake (kg ha^{-1})						
	N	**P**	**K**	**Fe**	**Zn**	**Cu**	**Mn**
T	46.34 ± 1.73	9.33 ± 1.60	18.04 ± 0.55	0.51 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.07 ± 0.02
T_2	80.02 ± 1.73	16.74 ± 1.05	35.04 ± 1.10	0.88 ± 0.02	0.12 ± 0.01	0.07 ± 0.02	0.13 ± 0.01
T_3	87.16 ± 1.12	19.24 ± 2.26	39.05 ± 1.10	0.96 ± 0.01	0.14 ± 0.02	0.08 ± 0.01	0.15 ± 0.02
T	62.55 ± 1.07	13.76 ± 1.76	30.86 ± 0.69	0.69 ± 0.02	0.10 ± 0.01	0.06 ± 0.02	0.09 ± 0.01
T_5	66.58 ± 1.22	15.77 ± 1.73	31.97 ± 0.65	0.73 ± 0.01	0.01 ± 0.01	0.06 ± 0.01	0.11 ± 0.02
T_6	66.18 ± 1.12	17.93 ± 1.78	32.45 ± 1.62	0.72 ± 0.02	0.10 ± 0.01	0.06 ± 0.02	0.11 ± 0.01
T_7	64.02 ± 0.83	16.54 ± 0.90	31.38 ± 1.62	0.70 ± 0.01	0.10 ± 0.02	0.06 ± 0.01	0.10 ± 0.02
CD (p=0.05)	**4.19**	**5.41**	**4.01**	**0.05**	**NS**	**NS**	**NS**

Note: For treatments detail see table 1.

Figure 1. Effect of inorganic fertilizer application on (A) Soil available N, (B) Soil available P (C) Soil available K (D) Soil available Fe .

Table 9. Effect of inorganic fertilizer application on nutrient uptake of maize stover.

T	Nutrient uptake (kg ha^{-1})						
	N	P	K	Fe	Zn	Cu	Mn
T_1	46.67 ± 1.86	6.48 ± 1.59	52.28 ± 1.85	0.84 ± 0.01	0.05 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
T_2	97.08 ± 1.35	19.42 ± 1.97	127.47 ± 1.20	1.82 ± 0.01	0.12 ± 0.01	0.04 ± 0.01	0.09 ± 0.01
T_3	109.13 ± 1.21	23.32 ± 1.57	146.43 ± 1.51	2.05 ± 0.01	0.15 ± 0.01	0.05 ± 0.01	0.11 ± 0.01
T_4	83.72 ± 1.39	12.60 ± 1.97	92.61 ± 2.11	1.59 ± 0.01	0.10 ± 0.01	0.03 ± 0.01	0.07 ± 0.01
T_5	96.50 ± 1.28	14.79 ± 1.76	107.38 ± 2.40	1.69 ± 0.01	0.11 ± 0.01	0.04 ±0.01	0.07 ± 0.01
T_6	99.30 ± 1.45	16.30 ± 1.80	110.16 ± 1.68	1.70 ± 0.01	0.11 ± 0.01	0.04 ± 0.01	0.08 ± 0.01
T_7	89.14 ± 1.48	13.71 ± 1.25	98.29 ± 1.56	1.64 ± 0.01	0.10 ± 0.01	0.03 ± 0.01	0.07 ± 0.01
CD (p=0.05)	4.79	5.61	5.85	0.05	0.04	NS	NS

Note: For treatments detail see table 1.

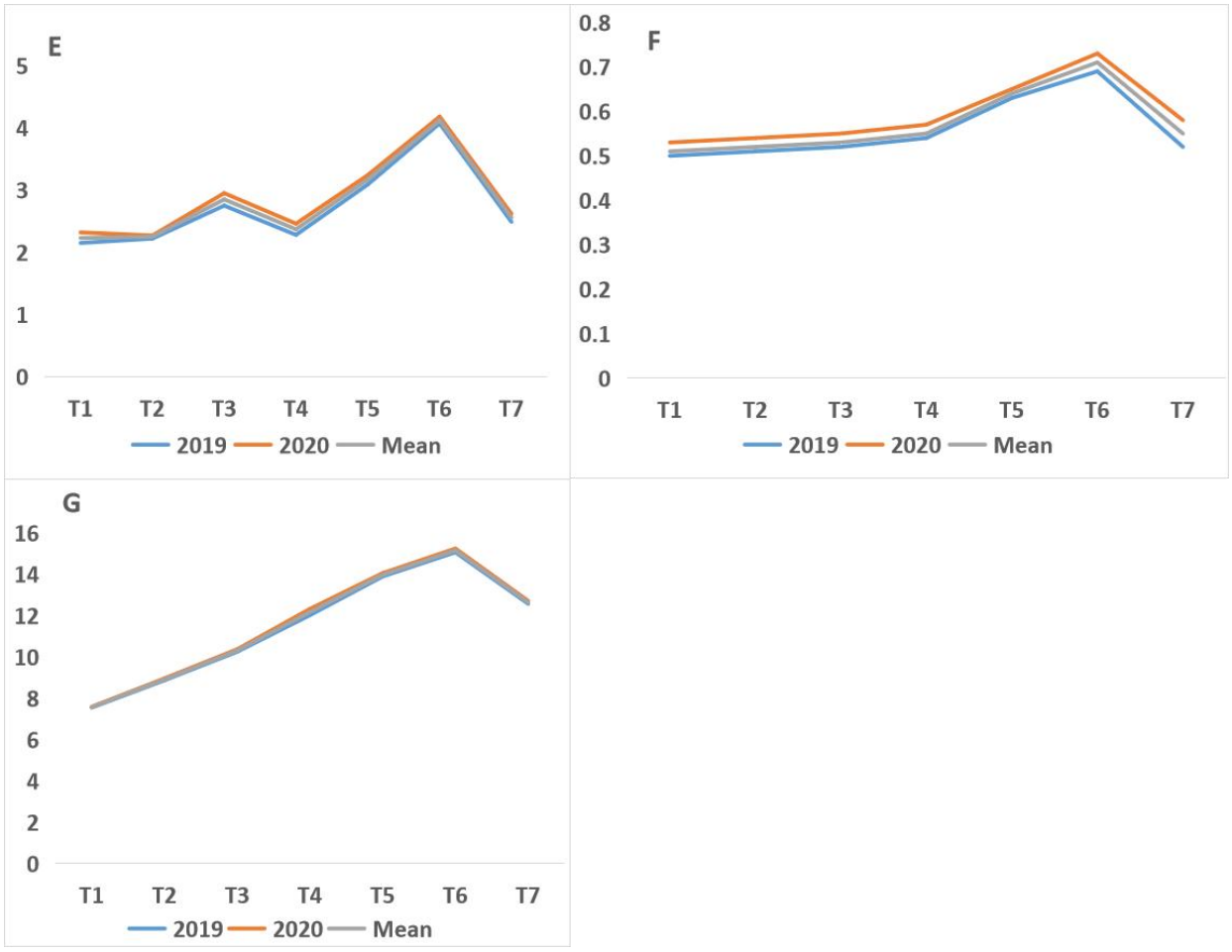

Figure 2. Effect of inorganic fertilizer application on (E) Soil available Zn (F) Soil available Cu (G) Soil Available Mn.

growth that results in increased nutrient uptake from the soil. However, increased nutrient content with dhaincha leaves overoptimistic may be due to the comparatively increased nutrient content in matured dhaincha leaves overoptimistic in comparison to other vermicomposts.

3.5.2 Nutrient uptake of maize grain and stover

The nutrient uptake of maize grain was computed by multiplying the nutrient content of grains with yield recorded in the particular treatment. Higher nutrient uptake was noted with INM treatment followed by RDF, different overoptimistic and control treatments (Table 8&9). The higher uptake of nutrients is attributed to higher yields along with higher nutrient content absorbed in these treatments.

4. Conclusion

The application of chemical fertilizers along with organic manure is growing popular among farmers due to growing awareness and enhanced productivity while maintaining soil fertility. In the present experiment, the maximum yield attributes were recorded with integrated nutrient management followed by recommended dose of fertilizer treatment, which lied at par to the neem leaves overoptimistic treatment and showed significant increase in comparison to other overoptimistic treatments. However, all the treatments showed better results in comparison to control. The combination of both was reported quite suitable because the joint action of FYM and chemical fertilizers leads to stabilized crop production and maintain greater productivity. Furthermore, when the organic and inorganic fertilizers are applied in combination, improves the N. P and K content as the process like decomposition and mineralization increases the nutrient availability in the soil. Further, nutrient losses also take with the chemical fertilizers' application. The organic fertilizers such as FYM and overoptimistic are slow releasing fertilizers. These fertilizers upon decomposition and mineralization improves the nutrient and organic carbon status of the soil and hence improves the crop growth and yield. The long-term experiments should be conducted to evaluate the improvement in crop growth and yield with the continuous application of organic fertilizers to the soil for further several years.

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ORIGINAL RESEARCH

Response of various cultivars of cucumber to different isolates of *Pseudoperonospora cubensis* (Berk et Curt.) Rostow under artificial epiphytotic conditions

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ABSTRACT: This research was conducted at the Department of Plant Pathology, University of Agriculture Peshawar during the 2011 growing season of the crop to determine the response of various cultivars of cucumber to different isolates of the downy mildew fungus *Pseudoperonspora cubensis* under artificial epiphytotic conditions. Five cucumber cultivars (Desi, Long Green, F1 hybrid, Dollar and Khyber) were tested for their response to infection by four different isolates of *Pseudoperonospora cubensis*. Significant differences ($p<0.05$) were found among the treated and control plants. F1 Hybrid and Dollar F1 were found to have the least disease severity. Among the isolates, isolate 4 caused the highest disease severity. In control plants, the disease severity was less. The interaction of cultivars and isolates was also significant in disease severity after thirty and forty days of inoculation.

KEYWORDS: Cucumber cultivars, downy mildew, *Pseudoperonospora cubensis* and isolates.

1. Introduction

Cucumber *(Cucumis sativus L.)* is the fourth most widely grown vegetable crop (Tatlioglu, 1993) in the world after tomato (*Lycopersicon esculentum* Mill.), cabbage *(Brassica oleracea var. capitata L.)* and onion *(Allium cepa L.).* Species of the Cucurbitaceae family are grown widely around the world as crops. The family is comprised of about 118 genera and 825 species that are primarily annual vines (Jeffrey, 1990). The genus *Cucumis* contains 52 species, of which *C. sativus* and *C. melo* are the two most economically significant (Ghebretinsae et al., 2007). In Pakistan, cucumbers are grown on 1,274 hectares producing 5,539 tonnes of fruits (MINFAL, 2008).

Cucurbit downy mildew is distributed widely worldwide (Palti and Cohen, 1980) and is one of the most destructive pathogens of cucurbits. It is the most important disease of cucumber, causing more than 50 % losses. The downy mildew influencing factors are environment and host. This disease has been reported from 70 countries, with the most severe outbreaks occurring in humid regions. Environmental conditions play a fundamental role in disease intensity (Cohen, 1977). Leaf wetness is critical for the disease to progress; if free moisture is not on the leaf, sporangia

will not germinate. Adequate leaf moisture is provided by rainfall, dew or irrigation. Ideal temperature for infection is 15° C (Thomas, 1977).

In cucumber, symptoms of downy mildew occur on the leaf blades. Infection first appears as small, water-soaked lesions on the underside of leaves, the lesions turn chlorotic and dark-colored spores form on the underside of the leaf. Chlorotic spots turn necrotic. Eventually, the entire leaf will become necrotic and die (Palti and Cohen, 1980; Chen et al., 2020). Downy mildew symptoms on cucumber will vary depending on its level of resistance (Shirley et al., 2022). The most resistant cucumber cultivars exhibited a hypersensitive response with small necrotic or chlorotic spots and limited sporulation (Chakraborty et al., 2022). The most susceptible will show many large chlorotic and necrotic lesions with abundant sporulation.

Studies on the host range of *P. cubensis* indicated that approximately 20 genera, including 50 species in the Cucurbitaceae, were there hosts. A total of 19 host species are in the genus *Cucumis* (Palti and Cohen, 1980; Lebeda, 1992a, and Lebeda and Widrlechner, 2003). In addition to cucumber, other economically important hosts of *P. cubensis* are melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai and squash (*Cucurbita* spp.) (Whitaker and Davis, 1962).

There are several strategies to manage the disease. These include cultural, chemical and planting resistant or tolerant varieties. Several fungicides have been found effective against the disease. These includes Alliette, Chlorothalonil and Ridomil gold (Sharma et al., 2003; Obradovic et al., 1992). However, these fungicides are expensive, hazardous to human beings, animals and plants. Moreover, protectant fungicides are less effective (Zitter et al., 1996 and Chaban et al., 1993). Hence, host resistance is an important alternative to minimize crop losses from *P. cubensis.* Some of the varieties have been found resistant, some tolerant while some susceptible to *P. cubensis* (Wehner and Shetty, 1997 and Zinati et al., 1987). In order to reduce the yield losses, it is essential to thoroughly test the resistance of locally available germplasms against *P. cubensis* and to pinpoint resistant or tolerant varieties.

The present study was carried out to evaluate the distribution of downy mildew of cucumber in district Nowshera, to evaluate cucumber cultivars for resistance to different isolates of *P. cubensis* and to assess the losses in yield components under artificial epiphytotic conditions.

2. Materials and methods

This pot experiment was conducted at the Department of Plant Pathology, Khyber Pakhtunkhwa Agricultural University, Peshawar during 2011 growing season of cucumber crop.

1. Severity of cucumber downy mildew in Nowshera

The survey was conducted in the cucumber growing regions to find out the severity of cucumber downy mildew in District Nowshera. Four locations (Tarujabba, Akora khattak, Peersabak and Jhangeera) were selected. Disease severities were assessed by

using the key (Table 1) ofJenkins and Wehner(1983). Disease severity was recorded in four fields in each location. Data on downy mildew severity was taken from four spots in each field. In each field, one meter square spot was selected and by visual observation and using the key, the data on cucumber downy mildew were recorded.

Table 1. Jenkins and Wehner (1983) disease severity key for downy mildew of cucumber.

Disease Severity Rating	Disease Severity Description
0	No foliar symptoms
1-2	Trace, 3-6 % of leaf area infected
3-4	Slight, 7-25 % of leaf area infected
5-6	Moderate, 26-75 % of leaf area infected
7-8	Advance, 76-94 % of leaf area infected
9	Plant dead, 97 % or more of leaf area infected

2. Pot experiment

In In the laboratory, infected leaves collected from four locations (Tarujabba, Peersabak, Akora khattak, Jhangeera) were soaked in sterilized distilled water and rubbed gently to dislodge sporangia. The sporangia concentration was determined with the help of a haemocytometer. The suspension was adjusted to $5x10^4$ sporangia/ml using sterilized distilled water (Vara et al., 1982). Nursery of five cucumber varieties (Desi, Long green, F_1 Hybrid, Dollar F_1 and Khyber) was raised in a screen house. A pot experiment was designed using 2 factors Completely Randomized (CR) design with three replications. There was a total of 25 treatments in each replication i.e. four isolates and one untreated check and five varieties. A total of 75 pots of uniform diameter (20cm) were selected. Then, farm yard manure (one-year-old), silt and clay were thoroughly mixed in the ratio of 1:1:1 (v/v). These 75 pots were filled with the medium and watered before transplantation. Cucumber seedlings at the 2 to 3-leaf stage were carefully uprooted and washed. The roots were inoculated by dipping in the spore suspension (5×10^4 sporangia/ ml) of *Pseudoperonospora cubensis* isolates as described by Vara et al., (1982) and transplanted into the pots. Pots were regularly checked and watered as required. Data were recorded on the following parameters;

2.1 Disease Severity (%)

Data on disease severity was recorded four times at an interval of ten days. The first data were recorded after ten days of inoculation according to the disease severity scale proposed by Jenkins and Wehner (1983).

2.2 Number of vines per plant

Data were recorded on the number of vines per plant for each variety. This was calculated before the senescence of the crop.

2.3 Vine Length (cm)

The length of the vines was measured for each treatment. This was done before the senescence of the crop.

2.4 Number of fruits per plant

Data on the number of fruits per plant were recorded. This was recorded by totaling the number of fruits in all picking.

2.5 Fruit yield (gm) per plant

Data on fruit yield were taken for all treatments in each replication. As this crop is harvested in picking, total yield was calculated by adding all selections. All the recorded data were pooled for statistical analysis using F-test and means were separated by the Least Significant Difference (LSD) test (Dana, 2001).

2.6 Statistical analysis

Analyses were carried and the significant means for various traits were separated with the application of LSD test.

RESULTS

1. Severity (%) of cucumber downy mildew in Nowshera

Data presented in Table 2 indicated significant differences ($p< 0.05$) of downy mildew severity among four locations (Peersabak, Jhangeera, Tarujabba and Akora Khattak). Maximum (54.3 %) disease severity was recorded in Jhangeera followed by Akora khattak (48.2 %). Minimum (19.7 %) was recorded in Peersabak. In five field highest (47.4 %) disease severity was found in field five and the lowest (22.6%) in field 2. Interaction of the locations and fields were also significantly different in downy mildew severity. In Peersabak, Jhangeera, Tarujabba, and Akora Khattak, the highest percentage of downy mildew was recorded in F_5 (76.0%), F_4 (83.0%), F_3 (44.5%), F_2 (39.5%) and F_1 (82.0%), respectively. While this was 12.5 (F_1), 4.8 (F_2), 15.8 (F_3), 17.3 (F_4) and 32.3% (F_5), respectively in the said locations (minimum).

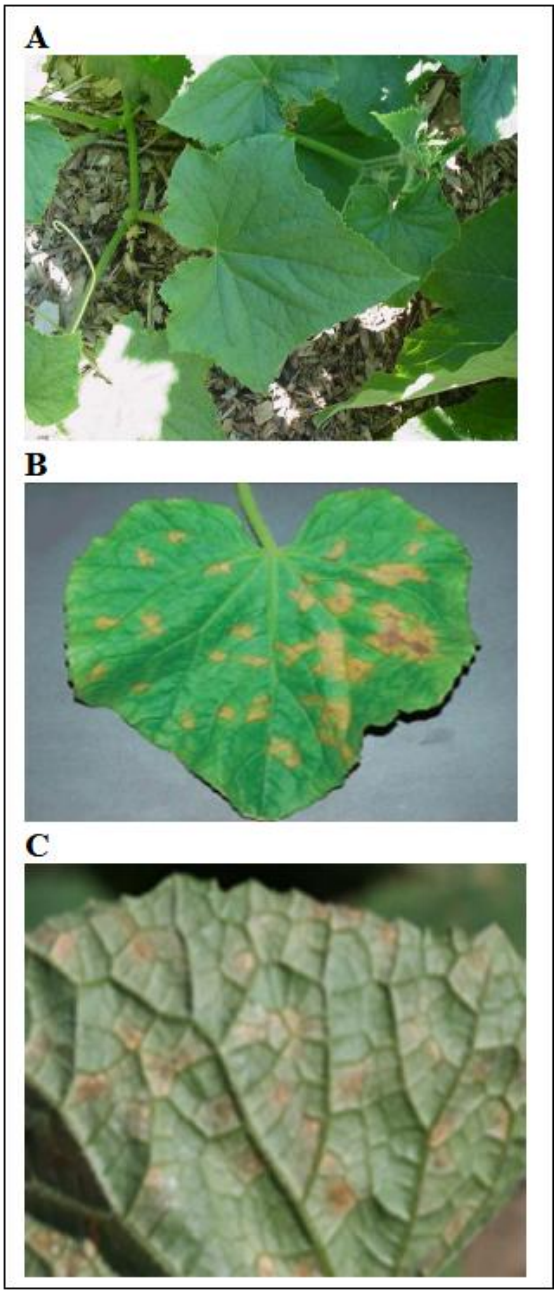

Figure 1. Symptoms of *Pseudoperonospora cubensis* on cucumber leaves. (A) Cucumber Leaf (Healthy), (B) Infected leaf, (C) Infected leaf.

2. Pot experiment

2.1. Disease severity (%)

Data recorded after ten days of inoculation of cucumber cultivars with different isolates showed significant differences in percent disease severity (Table 3). This was 2.7 to 2.9 % in different cultivars. The virulence of different isolates was 3.5 to 3.7 % with the highest of Peersabak isolate. However, the untreated check had no disease. Response of the cultivars to different isolates was also different. There was no disease on the untreated check. The cultivars responded 3.3-4.0, 3.3-3.7, 3.3-3.7 and 3.3- 3.7 % to different isolates (I_1, I_2, I_3 & I_4), respectively (Figure 1 & 2).

Disease severity data recorded after twenty days of inoculation of cucumber cultivars showed significant differences (Table 4) The down mildew severity on cultivars were 6.2 to 6.5%. The virulence of *P. cubensis* isolates on different cultivars was 7.4 to 7.9%. The cultivars which were not inoculated having 1.0% disease severity. The interaction of cultivars and isolates also had significant differences among themselves.

These were 1.0-8.3, 1.0-8.0, 1.0-7.7, 1.0-7.7 and 1.0-8.0% disease severity on Desi , Long green, F_1 Hybrid, Dollar F_1 and Khyber, respectively. The effect of different isolates (I_1, I_2, I_3& I_4) on cultivars were 7.3-7.7, 7.3-7.7, 7.7-8.0 and 7.7-8.3%, respectively.

Data on percent disease severity after 30 days of inoculation showed significant differences among the cultivars and the isolates (Table 5). Among the various isolates (Tarujabba, Peersabak, Akora Khattak and Jhangeera), the least (2.1%) disease severity was in control followed by Tarujabba (24.5%), Peersabak (25.1%), Akora khattak (25.1%) and Jhangeera (25.3%).

The various cucumber cultivars tested for disease severity (%) to different isolates differed significantly from each other. Cultivars Long green (22.5%), Khyber (22.4%), and Desi (22.0%) respectively gave no significant differences in disease severity indicating that they are equally susceptible to the disease. The least disease severity was recorded in F_1 Hybrid (17.4%) and Dollar F_1 (17.7%). Likewise, significant differences in disease severity (%) were seen as a result of interaction between different cultivars and isolates. Although disease severity was observed in all the combinations however, differences were significant. Variety Long green and Khyber showed the highest (26.7%) disease severity to isolate Tarujabba. Desi variety showed 26.0% disease severity. The least disease severity was recorded in Dollar F_1 (21.3%) and F_1 Hybrid (21.7%). On the other hand the highest disease severity was recorded in Long green (28.0%) followed by Khyber (27.3%) and Desi (26.7%) subjected to Peersabak isolate. F_1 Hybrid (21.1%) and Dollar F_1 (22.3%) showed the minimum disease severity. The varieties Long green and Desi showed the highest (27.7%) disease severity followed by Khyber (27.3%) in response to isolate Akora Khattak. while the lowest disease severity in F_1 Hybrid (21.3%) and Dollar F_1 (21.7%) of Peersabak and Tarujabba isolates. Variety Long green and Khyber showed the highest (28.3%) disease severity against the isolate Jhangeera. However, Desi showed 27.7%. F_1 Hybrid and Dollar F_1 gave the same and minimum disease severity (21.0%).

Data (Table 6) recorded after 40 days of inoculation showing significant differences ($p<0.05$) in disease severity. Among the 4

isolates, the highest disease severity was in a Jhangeera (60.5%) and the minimum disease severity in control (5.6%) followed by isolate Taru jabba (58.9%), while isolate Peersabak (59.7%) and isolate Akora Khattak (60.1%) showed no significant differences in disease severity. The least disease severity (29.1%) and (29.6%) in F_1 hybrid and Dollar $F_{1,,}$ respectively. The highest disease severity (63.5%) was in Khyber. No significant differences were found in Desi (61.4%) and Long green (61.3%), respectively. The interaction was also significant (p<o.o5). There were no significant differences among varieties (Desi, Long green, F_1 Hybrid, Dollar F_1 and Khyber) in control. Least disease severity (5.0%) was in Long green and F_1 Hybrid and highest disease severity in Khyber (6.7%) in control. The highest disease severity in Khyber (77.0%) followed by Desi (75.0%) and Long green (74.3%) to isolate Tarujabba. While least disease severity was in F_1 Hybrid (33.7%) and Dollar F_1 (34.7%). They showed no significant differences. Variety Khyber (76.7%) showed highest disease severity to isolate Peersabak. There were no significant differences in Desi (75.3%) and Long green (76.0%). There were also non-significant differences in F_1 hybrid and Dollar F_1 (35.3%) showed least disease severity (%). Variety Khyber showed highest (79.3%) disease severity to isolate Akora Khattak. There were non-significant differences in variety Desi and Long green (75.0%). Least disease severity was in variety F_1 Hybrid (35.0%) and Dollar F_1 (36.0%). Disease severity was high in Khyber (77.7%) to isolate Jhangeera followed by Desi (76.3%) and Long green (76.0%). Non-significant differences in F_1 Hybrid (36.7%) and Dollar F_1 (36.0%) having least disease severity.

2.2. Number of vines

Data in Table 7 indicated main effect of isolates exhibited no significant effect on no of vines. Mean number of vines ranged from 1.9 to 2 .1 in 4 isolates. Maximum (2.8) no of vines recorded in control. However, in main effect of varieties F_1 Hybrid and Dollar F_1 had non-significant and maximum (2.7) no of vines. No of vines was minimum (1.7) in Long green and Khyber. The interaction was significant. In control, the maximum number of vines were in Dollar F_1 (3.7) and minimum in Khyber (2.3), Desi (2.3) and Long green (2.3), respectively. Variety Dollar F_1 (2.7) produced maximum number of vines to isolate Tarujabba. While it was minimum (1.7) in Long green and Khyber. F_1 Hybrid and Dollar F_1 produced maximum (2.3) number of vines when infected with isolate 2 (Peersabak). While it was minimum (1.7) in Desi , Long green and Khyber. There were non significant difference in F_1 Hybrid and Dollar F_1 showed maximum (2.7) number of vines to isolate 3 (Akora Khattak) and minimum in Khyber (1.3) to isolate 3 (Akora Khattak). Number of vines were maximum in variety F_1 Hybrid (2.7) to isolate 4 (Jhangeera) and variety Long green (1.3) showed minimum to that isolate. The number of vines of non inoculated cultivars were 2.3 to 3.7 with highest (3.7) in Dollar F_1.

2.3. Vine length (cm)

Non significant differences (P > 0.05) were observed among various isolates (Table 8) .

Table 2. Severity (%) of *Pseudoperonospora cubensis,* the cause of cucumber downy mildew in Nowshera.

Locations	Fields (F)					Mean
	F_1	F_2	F_3	F_4	F_5	
Peersabak	16.5g	15.8g	16.5g	17.3g	32.3g	19.7c
Jhangeera	60.8ef	39.5b	44.5cde	83.0c	43.8a	54.3a
Tarujabba	12.5gh	4.8h	15.8e	33.8ef	37.5cdef	20.9cdef
Akora khattak	82.0a	30.3f	16.3g	36.3def	76.0a	48.2b
Mean	42.9	22.6c	23.3c	42.6b	47.4a	35.7

LSD value for location (L) = 3.6, LSD value for field (F) = 4.0, LSD value for F X L = 8.0
CV (%) = 15.9

Table 3. Effect of different isolates of *Pseudoperonospora cubensis* on different cucumber varieties after ten days of inoculation.

Isolates	Varieties					Mean
	Desi	Long green	F_1 Hybrid	Dollar F_1	Khyber	
Control (I_0)	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b
Tarujabba (I_1)	3.7a	3.3a	3.7a	3.3a	3.7 a	3.5a
Peersabak (I_2)	4.0a	3.7a	3.7a	3.3a	3.7a	3.7a
Akora khattak (I_3)	3.3a	3.3a	3.3a	3.7a	3.7a	3.5a
Jhangeera (I_4)	3.7a	3.7a	3.7a	3.3a	3.3a	3.5a
Mean	2.9a	2.8a	2.9a	2.7a	2.9a	2.3

LSD value for isolates (I) = 0.4; LSD value for varieties (V) = 0.4; LSD value for I x V = 0.0
CV (%) = 19.1 * Means followed by different letter(s) are significantly different from one another at 5% level of significance.

Table 4. Effect of different isolates of *Pseudoperonospora cubensis* on different cucumber varieties after twenty days of inoculation.

Isolates	Varieties					Mean
	Desi	Long green	F_1 Hybrid	Dollar F_1	Khyber	
Control (I_0)	1.0 b	1.0b	1.0b	1.0b	1.0b	1.0b
Tarujabba (I_1)	7.7 a	7.3a	7.3a	7.3a	7.3a	7.4a
Peersabak (I_2)	7.3 a	7.7a	7.7a	7.7a	7.3a	7.5a
Akora khattak (I_3)	8.0 a	8.0a	7.7a	7.7a	7.7a	7.8a
Jhangeera (I_4)	8.3 a	7.7a	7.7a	7.7a	8.0a	7.9a
Mean	6.5 a	6.3a	6.3a	6.3a	6.3a	6.3

LSD value for isolates (I) = 0.7; LSD value for varieties (V) = 0.7; LSD value for I x V = 1.7
CV (%) = 16.0

Table 5. Effect of different isolates of *Pseudoperonospora cubensis* on different cucumber varieties after thirty days of inoculation.

Isolates	Varieties					Mean
	Desi	Long green	F_1 Hybrid	Dollar F_1	Khyber	
Control (I_0)	2.0d	2.0d	2.0d	2.0d	2.3d	2.1b
Tarujabba (I_1)	26.0b	26.7ab	21.7c	21.3c	26.7ab	24.5a
Peersabak (I_2)	26.7ab	28.0ab	21.0c	22.3c	27.3ab	25.1a
Akora khattak (I_3)	27.7ab	27.7ab	21.3c	21.7c	27.3ab	25.1a
Jhangeera (I_4)	27.7ab	28.3a	21.0c	21.0c	28.3a	25.3a
Mean	22.0a	22.5a	17.4b	17.7b	22.4a	20.4

LSD value for isolates (I) = 1.03; LSD value for varieties (V) = 1.03; LSD value for I x V = 2.3 CV (%) = 6.9 ; * Means followed by different letter(s) are significantly different from one another at 5% level of significance.

Table 6. Effect of different isolates of *Pseudoperonospora cubensis* on different cucumber varieties after forty days of inoculation.

Isolates	Varieties					Mean
	Desi	Long green	F_1 Hybrid	Dollar F_1	Khyber	
Control (I_0)	5.3e	5.0e	5.0e	6.0e	6.7e	5.6e
Tarujabba (I_1)	75.0bc	74.3c	33.7d	34.7d	77.0abc	58.9b
Peersabak (I_2)	75.3bc	76.0bc	35.3d	35.3d	76.7abc	59.7abc
Akora khattak (I_3)	75.0bc	75.0bc	35.0d	36.0d	79.3a	60.1ab
Jhangeera (I_4)	76.3abc	76.0bc	36.7d	36.0d	77.7ab	60.5a
Mean	61.4b	61.3b	29.1c	29.6c	63.5a	48.9

LSD value for isolates (I) = 1.5; LSD value for varieties (V) = 1.5; LSD value for I x V = 3.3 CV (%) = 4.1

Table 7. Effect of different isolates of *Pseudoperonospora cubensis* on number of vines of different cucumber cultivars.

Isolates	Varieties					Mean
	Desi	Long green	F_1 Hybrid	Dollar F_1	Khyber	
Control (I_0)	2.3cd	2.3cd	3.3ab	3.7a	2.3cd	2.8a
Tarujabba (I_1)	2.0cde	1.7de	2.3cd	2.7bc	1.7de	2.1b
Peersabak (I_2)	1.7de	1.7de	2.3cd	2.3cd	1.7de	1.9b
Akora khattak (I_3)	1.7de	1.7de	2.7bc	2.7bc	1.3e	2.0b
Jhangeera (I_4)	1.7de	1.3e	2.7bc	2.3cd	1.7de	1.9b
Mean	1.9b	1.7b	2.7a	2.7a	1.7b	2.1

LSD value for isolates (I) = 0.4; LSD value for varieties (V) = 0.4; LSD value for I x V = 0.98 CV (%) = 27.9 * Means followed by different letter(s) are significantly different from one another at 5% level of significance.

Table 8. Effect of different isolates of *Pseudoperonospora cubensis* on vine length (cm) of different cucumber cultivars.

Isolates	Varieties					Mean
	Desi	Long green	F_1 Hybrid	Dollar F_1	Khyber	
Control (I_0)	129.3a	113.3b	97.0cde	102.7c	101.7cd	108.8a
Tarujabba (I_1)	87.7fgh	87.0fgh	89.0efgh	86.7fgh	88.0fgh	87.7b
Peersabak (I_2)	93.7defg	88.0fgh	91.0efgh	84.7h	85.7gh	88.6b
Akora khattak (I_3)	94.0c-g	85.7gh	89.0efgh	88.0fgh	87.0fgh	88.7b
Jhangeera (I_4)	95.3cdef	88.7efgh	89.3efgh	87.7fgh	88.7efgh	89.9b
Mean	100.0a	92.5b	91.1 b	89.9b	90.2b	92.7

LSD value for isolates (I) = 3.98; LSD value for varieties (V) = 3.98; LSD value for I x V = 8.9
CV (%) = 5.9

Table 9. Effect of different isolates of *Pseudoperonospora cubensis* on number of fruits per plants of different cucumber cultivars.

Isolates	Varieties					Mean
	Desi	Long green	F_1 Hybrid	Dollar F_1	Khyber	
Control (I_0)	5.7de	6.0de	8.3a	7.7ab	5.3ef	6.6a
Tarujabba (I_1)	3.7gh	3.3gh	7.3abc	6.7bcd	4.3fg	5.1b
Peersabak (I_2)	3.3gh	3.3gh	7.3abc	6.3cde	3.7gh	4.8b
Akora khattak (I_3)	3.7gh	3.3gh	7.7ab	6.3cde	3.7gh	4.9b
Jhangeera (I_4)	3.0h	3.3gh	7.3abc	6.3cde	3.3gh	4.7b
Mean	3.9c	3.9c	7.6a	6.7b	4.1c	5.2

LSD value for isolates (I) = 0.5; LSD value for varieties (V) = 0.5; LSD value for I x V = 1.02
CV (%) = 11.9 * Means followed by different letter(s) are significantly different from one another at 5% level of significance.

Table 10. Effect of different isolates of *Pseudoperonospora cubensis* on yield per plant (gm) of different cucumber cultivars.

Isolates	Varieties					Mean
	Desi	Long green	F_1 Hybrid	Dollar F_1	Khyber	
Control (I_0)	606.7gh	626.7fgh	1000.0a	839.3b	563.3h	727.2a*
Tarujabba (I_1)	381.7ij	381.7ij	791.7bcd	728.3cde	441.7i	545.0b
Peersabak (I_2)	345.3j	384.3ij	806.3bc	693.3efg	382.0id	522.3b
Akora khattak (I_3)	376.3ij	380.0ij	800.0bcd	711.7def	388.0ij	531.2b
Jhangeera (I_4)	313.3j	377.3ij	801.7bcd	681.7efg	348.7ij	504.5b
Mean	404.7c	430.0 c	839.9a	730.9b	424.7c	566.0

LSD value for isolates (I) = 42.1; LSD value for varieties (V) = 42.1; LSD value for I x V = 94.2
CV (%) = 10.2 * Means followed by different letter(s) are significantly different from one another at 5% level of significance.

The highest (89.9 cm) in isolate 4 (Akora Khattak) while plants exhibited the least vine length (87.7 cm) in isolate 1 (Tarujabba). Vine length of five cultivars were 89.9-100.0 cm with highest (100 cm) in Desi and lowest (89.9 cm) in Dollar F_1.

The interaction was also significant and gave the differences in vine length of test cultivars under various isolates. F_1 Hybrid gained the highest (89.0 cm) vine length to isolate I_1 (Tarujabba). The vine length of Desi (87.7 cm), Long green (87.0 cm), Khyber (88.0 cm), Dollar F_1 (86.7 cm) and F_1 Hybrid (89.0 cm) were non significant among themselves. Likewise, the highest value of vinelength was obtained in Desi (93.7cm) to isolate 2 (Peersabak) followed by F_1 hybrid (91.0 cm). While it was least (84.7cm) in Dollar F_1. Desi (94.0 cm) showed the highest vine length to isolate 3 (Akora Khattak) followed by F_1 Hybrid (98.0cm), while the least vine length in Long green (85.7cm) . Moreover, the plants inoculated with I_4 (Jhangeera) produced vine length by 87.7 to 95.3 cm with highest (95.3 cm) in cultivar Desi. The vine length of non inoculated cultivars were 97.0 -129.3 cm with highest (129.3 cm) of cultivar Desi.

2.4. Number of fruits per plant

Significant difference ($P<0.05$) were observed among main effects in various cultivars (Table 9). Highest (7.6) number of fruits per plant was observed in F_1 Hybrid followed by Dollar F_1 (6.7). Plants exhibited the least mean number of fruits (3.9) in cultivars Desi and Long green. There were non-significant differences in various isolates. The highest (6.6) value of number of fruits per plant were in control followed by (5.1) isolate I_1 (Tarujabba). The interaction was significant and gave differences in number of fruits per plants. Among the cultivars, the highest number of fruits per plant in control of F_1 Hybrid was 8.3 followed by Dollar F_1 (7.7). While it was minimum in Khyber (5.3) in isolate I_1 (Tarujabba). Number of fruits per plant in Desi and Long green were 3.3 for isolate 2 (Peersabak). F_1 Hybrid showed highest (7.3) number of fruits per plant followed by Dollar F_1 (6.3). No significant differences between Desi (3.3) and Long green (3.3) were shown. Maximum number of fruits per plant were produced by F_1 Hybrid (7.7) followed by Dollar F_1 (6.3) to isolate 3 (Akora Khattak) and minimum by Long green (3.3). Likewise isolate 4 (Jhangeera) showed the same results that highest (7.3) number of fruits per plant were of F_1 Hybrid plants followed by Dollar F_1 (6.3) and minimum in Desi (3.0).

2.5. Yield per plant (gm)

Data in Table 10 indicated significant differences occurred in yield per plant (gm) on various isolates. Highest yield (727.2 gm) was obtained in check plants. F_1 Hybrid gave maximum (1000.0 gm) yield per plant followed by Dollar F_1 (839.3 gm) while minimum yieldwas obtained in Khyber (563.3 gm). However, out of four isolates Tarujabba (I_1) gave the highest (545.0 gm) followed by isolate Peersabak (522.3 gm). Minimum (504.5 gm) yield was obtained in Jhangeera (I_4). On contrary significant differences ($p<0.05$) in yield per plant was recorded in various test cultivars. F_1 hybrid gave the highest (839.9 gm) yield per plant. It was followed by Dollar F_1 (730.9 gm). Minimum was recorded in Desi (404.7 gm). Differences

in yield were significant in interaction too. F_1 Hybrid gave the highest yield (791.7 gm), followed by Dollar F_1 (728.3 gm) to Tarujabba (I_1). Non significant differences were found in Desi (381.7gm) and Long green (381.7 gm) which were minimum. Likewise, highest yield (806.3 gm) were found in F_1 Hybrid followed by Dollar F_1 (693.3 gm) to Peersabak (I_2). Minimum yield was recorded in Desi (345.3 gm). Variety F_1 Hybrid gave highest (800.0 gm) followed by Dollar F_1 (711.7 gm) to Akora Khattak (I_3) and minimum was recorded in Desi. F_1 Hybrid and Dollar F_1 gave the highest yield (801.7 gm) and (681.7 gm) to isolate Jhangeera (I_4), respectively.

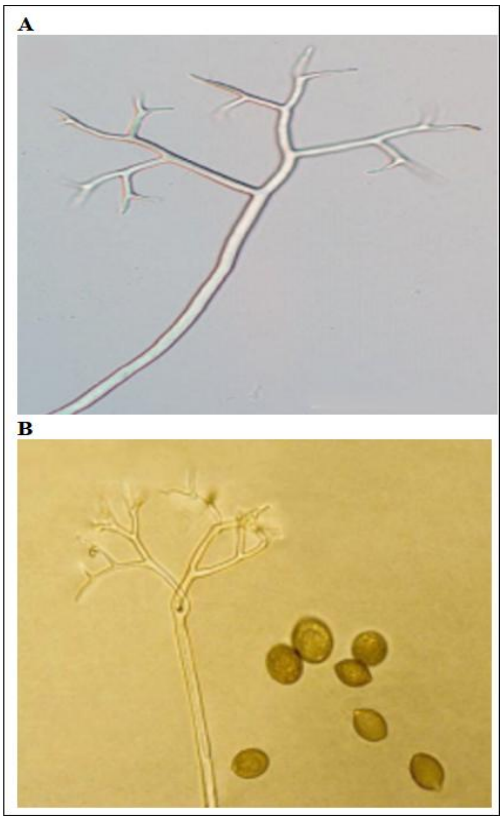

Figure 2. Structure of (A) Sporangia and (B)sporangiaphore of Pseudoperonospora cubensis.

4. DISCUSSION

Downy mildew has been observed to cause severe yield losses to cucumber in Khyber Pakhtunkhwa. The disease not only reduces the yield but also adversely affects the quality in the form of misshapen cucumber fruits. Such fruits fetch low price in the market. On the other hand, low yield are obtained when the photosynthetic area are badly damaged by the *Pseudoperonospora cubensis.* However, the rapid increase in fungicide prices, their less availability in the market and the ignorance of the farmers about their proper use or the use of non chemical methods have made downy mildew control more difficult. Keeping in view these points, this project research was aimed to investigate the variability among the different isolates for their virulence. The infestation of *P. cubensis* were highest in Jhangeera followed by Akora Khattak and lowest in Peersabak. Variability in disease severities were also found within the locations too. This might be due to virulence of the pathogen and favorable environmental conditions including sowing of susceptible varieties and monoculturing of the crop (Wehner and Sheity 1997, Nischit 2002 and Salati, 2010). In the present study, five cucumber cultivars (Desi, Long green, F_1 Hybrid, Dollar F_1 and Khyber) being tested for their response to 4 isolates that were collected from different locations (Tarujabba, Peersabak, Akora Khattak and Jhangeera) from Nowshera District.

Responses of the different varieties were not much variable to different isolates of *Pseudoperonospora cubensis.* With the passage of time, variability was disclosed by different varieties to those four isolates. After

ten days and twenty days inoculation there were non significant differences, however at thirty days the cultivars Long green, Khyber and Desi showed maximum disease severity indicating that these cultivars were highly susceptible and minimum disease severity were found in F_1 Hybrid and Dollar F_1 that showed these cultivars were moderately resistant but not immune to *Pseudoperonospora cubensis*. It was also reported that F_1 Hybrid was considered to be less susceptible to downy mildew (*Pseudoperonospora cubensis*) of cucumber. After forty days of infection, there were significant differences among the isolates and cultivars. Among the cultivars, F_1 Hybrid and Dollar F_1 were moderately resistant and showed minimum disease severity. Khyber showed high susceptibility followed by Long green and Desi. Among the four isolates, Jhangeera (I_4) was more aggressive followed by Akora Khattak, Peersabak and Tarujabba. In control there was least disease severity.

Maximum number of vines was recorded in F_1 Hybrid and Dollar F_1 while minimum in Long green and Khyber which was due to high infestation of the disease as compared to the infestation of different isolates. The control plants produced the maximum number of vines due to less attack of the disease. Maximum number of fruits per plant was found in F_1 Hybrid followed by Dollar F_1 and minimum fruits were recorded in Desi and Long green among the four isolates. Maximum numbers of fruits were obtained from Tarujabba isolate which were not much aggressive. They also produced maximum yield per plant. The maximum vine length (cm) was recorded in Desi. Minimum vine length recorded in Dollar F_1 followed by Khyber. However there was no significant difference found in four isolates. Least vine length recorded in Tarujabba isolate (I_1) and maximum vine length recorded in Jhangeera isolates (I_4). *Pseudoperonospora cubensis* is responsible for low yield parameters. This reduction was reported up to 80% (Lebeda and Schwinn, 1994; Lebeda and Urban, 2007).

Fruit yield is an important criterion to check and compare the genetic potential of different cultivars. Results showed that yield per plant was statistically different among the cultivars. Maximum fruit yield produced by F_1 Hybrid followed by Dollar F_1. This was due to less disease severity which contribute towards the final yield. Minimum yield per plant were recorded in Desi followed by Khyber and Long green. Crisswell *et al* (2008) and Crisswell & Wehner (2008) also reported similar results. Mean ratings for downy mildew foliar resistance of cucumber accessions in the germplasm were studied in North Carolina too (Adam, 2010). Among the four isolates there were non significant differences. Maximum yield per plant was found in Tarujabba because they were not aggressive and minimum in Jhangeera. The interaction of isolates with different cucumber cultivars resulted the difference in yield and yield components cultivars F_1 Hybrid and Dollar F_1 showed best results for having less disease severity and high yield per plant. Akora Khattak (I_3) and Jhangeera (I_4) were highly aggressive and they reduced the yield per plant as compared to other isolates (Adam, 2010).

5. CONCLUSIONS

The mean cucumber downy mildew severities were 19.7 to 54.3% with highest

(54.3%) in Jhangeera. Among the fields of different locations (interaction of locations and fields), it was 4.8 to 83.0%.

1. Mean cucumber downy mildew severity was 58.9 to 60.5% with maximum (60.5%) by Jhangeera isolates after 40 days of inoculation. Mean severity of disease of different isolates was 29.1 to 63.5% with minimum on variety F1 Hybrid. The interaction of isolates and varieties revealed that it was 33.7 to 79.3% with minimum in F1 Hybrid. The overall performance of cultivars F1 Hybrid was best having less disease severity and highest yield and yield components than the other cultivars.
2. Further systematic research for the hunt of the source of resistance against the cucumber downy mildew disease in different agro-ecological zones of Khyber Pakhtunkhwa is recommended.

Authors Contributions:
A.R and H.K conceived the main idea of research and wrote the manuscript. F.R and M revised the manuscript and provided suggestions. In addition HK and AR assessed and analyzed the data, and performed data collection. All authors have read and agreed to the published version of the manuscript.

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Competing interests
The authors declare no competing interests.

Data Availability statements: The data presented in this study are available on request from the corresponding author.

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ORIGINAL RESEARCH

Analysis of Apricot Germplasm through Phenotypic Traits Under the Agro-Climatic Condition

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ABSTRACT: This study was conducted to evaluate different apricot germplasms on the bases of phenotypic traits at the Agricultural Research Institute Mingora, Swat, during the year 2016. The experiment was laid out in a completely randomized design (CRD). We choose various traits of six different varieties of apricot, i.e., Protici, Vitilo, Begali, Shernabi, Swat Selection and Luizet, which are collected from diverse agro-ecological zones were evaluated to ascertain the extent of genetic diversity and assess geographical heterogeneity among these varieties. Data on different quantitative and qualitative traits such as number of fruits kg^{-1}, total soluble solids, fruit color, kernel taste, and stone nature were recorded through physical and biochemical tests. The variety Luizet produced the largest size fruit with an average of 17.33 fruits kg^{-1}. Whereas, the variety Begali produced the smallest size fruits with an average of 54 fruits kg^{-1}. The maximum total soluble solids (18.06 oBrix) were recorded in the variety Begali and Luizet followed by the variety Vitilo (17.36 ^{o}Brix). Whereas the least amount of TSS were recorded in the variety Swat selection (13.2 ^{o}Brix). The fruits of Shernabi, Swat selection and Luizet had a uniform yellow color. However, fruits of other varieties were greenish to yellowish. Furthermore, free stones were most frequent in the fruits of Protici, Vitilo, Begali, Swat Selection and Shernabi, whereas, Luizet had semi-cling stones. Our results suggest that the variety Luizet is the best in terms of fruit size, TSS, fruit color and kernel taste as compared to the other tested varieties and is recommended for cultivation under the agro-climatic condition of Swat.

KEYWORDS: Apricot, climate, germplasm, phenotypic-traits, fruit quality

1. Introduction

Apricot (*Prunus armeniaca*) belongs to family Rosaceae, and sub-family Prunoideae. According to botanical classification the section Armeniace consist of five species i.e., P. armeniace (common apricot), P. holosericea (Tibetan apricot), P. mume (Japenese apricot), P. brigantiaca (Alpine plum), and P. dasycarpa (black apricot) out of these P. armeniace is the most cultivated apricot (Rehder 1927; Rehder 1949; yilmaz, Kargi et al., 2012; Khadivi-Khub and Khalili 2017). Apricot cultivars are classified into four eco-geographical groups such as Irano-Caucasian, Central Asian, Dzhungar-Zailing

and European (Kostina 1960; Halász, Pedryc et al., 2010). However, Apricot (*Prunus armeniaca* L.) is an important stone fruit belong to the family Rosaceae, and mostly grown in the temperate climate regions of the world. *P. armeniaca* L. trees were mostly originated from Central Asia and China. It is reported that apricot is mostly originated specially from Xinjiang province of China (Maghuly, Fernandez et al., 2005; Yuan, Chen et al., 2007). There are a lot of apricot germplasm resources available across the world (Mehlenbacher, Cociu et al., 1991; Tian-Ming, Xue-Sen et al., 2007). Mostly apricot producing countries are Italy, France, Spain, Algeria, Morocco, Syria, Iran, Turkey, Uzbekistan, Afghanistan, USA and Pakistan which contribute approximately 80% of world apricot (Milosevic 2011; Zhebentyayeva, Ledbetter et al., 2012; Maryam, Rafi et al. 2020). According to FAO (2002) reported that Turkey has the major apricot producing and exporting country in the world. Globally, various research experiments were laid down to promote and enhance the production of high quality apricots (Vachun 1995). According to Anon (1998) apricot is one of the most important attractive, nutritious, delicious and common fruit of northern Pakistan (Hussain, Yasmin et al., 2010; Nadeem, Ishaq Javed et al., 2012; Karatas, Ercisli et al., 2021).

Apricot is a very significant fruit crop with numerous health advantages, which has increased its commercial benefits, particularly in the temperate zone. Meanwhile, due to its high vitamin and nutrient content it is quite popular with consumers. The customers are highly interested in high quality of apricots i.e., flavor, scent, and particularly their sugar level, which is one of the most distinguished quality feature (Ruiz and Egea 2008; Khadivi-Khub and Khalili 2017). The variety of apricot cultivars demonstrate the fruits of high degree of climatic adaptability. According to Ruiz and Egea, (2008) and Khadivi-Khub et al. (2017), the diversity of fruit cultivars allows the production of fruits with the best qualities, including high sugar contents, flavorful flesh, scent, significant size, attractive colour, and a lengthy harvesting time (Ruiz and Egea 2008, Khadivi-Khub and Khalili 2017). According to Leccese et al. (2007), it is a nutrient-rich source of carbohydrates, minerals, fibers, bioactive phytochemicals, and vitamins A, C, thiamine, niacin, riboflavin, and pantothenic acid (Leccese, Bartolini et al., 2007; Singh 2020). Carotenoids, phenolics, and antioxidants are among the phytochemicals that are significant due to their biological importance (Lichou, Jay et al., 2003; Fatima, Bashir et al. 2018).

Additionally, the average size of the apricot fruit is about 5cm in diameter, and it hold one large seed (Hussain, Yasmin et al., 2010; Sumonsiri and Barringer 2014). This delicious fruit trees are grown from plain to altitude area of about 3000 meters' height. It is used for the preparation of many useful products such as jam and nectar. Apricot is preserved mainly by conventional method i.e., sun drying without the use of chemicals in northern areas. Meanwhile, fresh fruits enter the market by the end of May and remains there through September, dried fruit is accessible all the year (Faqir, Saeed et al. 2004). According to PAR (Agriculture Education portal), Apricot are mostly grown in the northern areas of Pakistan. According

to statistical report of FAO, 2012, Pakistan is rated 6th position in terms of apricot production. Pakistan horticulture development and export board reported that in Baluchistan province especially the region named Killa Saifullah contributed about 60% of apricot production. Another report highlighted that approximately 60 apricot varieties are grown in the northern areas of Pakistan. Some of them are Halman, Karfo, Chuli, Marghulam, and Shara karfa (Alam 1990; Rana, Moeen et al., 2021). Pakistan is famous for the production of best apricot varieties across the world. However, various report shows that about 180 apricot varieties are found in Pakistan (Waseem, Naqvi et al., 2021). Some other varieties are Red flesh early, Old cap, Charmaghz, Moorpark, Nari, Shakarpara, Kassel Bright, Sarda, Budgher, Travet, Swat selection etc (Ali, Masud et al., 2011).

Furthermore, the annual production of apricot in Pakistan is 197.2 tones in which 8.3% is produced in Khyber Pakhtunkhwa (KPK). The supreme quality apricot is produced by those genotypes which grown at the upper altitude due to this reason greater quality of apricot is produced in the northern areas of Khyber Pakhtunkhwa for example in Swat, Manshera, and Hangu (Akhtar, Akmal et al., 2013). The current project was designed to investigate different verities of apricot i.e. Protici, Vitilo, Begali, Shernabi, Swat Selection and Luizet on the bases of phenotypic traits, for their quality and production under the agro-climatic conditions of Swat.

2. Material and methods

2.1 Experimental deign and location

Apricot germplasm orchard were currently established at Agriculture Research Institute Mingora, Swat by collecting apricot samples from various localities. There are six different germplasms selected for the current study i.e., Protici, Vitilo, Begali, Shernabi, Swat Selection and Luizet. The germplasm were evaluated for various traits during the course of this study.

2.2 Data collection

Apricot is an important medicinal crop plants of Khyber-Pakhtunkhwa especially of district Swat. The survey were carried out during the year of 2016. The data were collected on various parameters of the apricot such as fruit colour, kernel test, number of fruits per kilogram, nature of stones and total soluble solids.

2.2.1 Fruit colour

Fruit colour was observed on physical appearance which play an important role in the quality of fruits. Fruit color was measured through visual observation by the scale of 10 scoring method such as yellow = 0-2, yellowish = 3-4, white = 5-6, white with pink blush = 7-8, yellow with red blush = 8-9.

2.2.2 Kernel taste

Kernel test was taken from apricot fruit randomly and the taste of each kernel was checked for its sweetness and bitterness.

2.2.3 Number of fruits per kilogram

One-kilogram fruit sample was taken in each treatment per replication for each variety and the number of fruits was counted and recorded**.**

2.2.4 Nature of stone

For determining nature of stone, Apricot fruits were randomly taken from each genotype to observe the nature of stone whether it is free stone, semi-cling stone or cling stone.

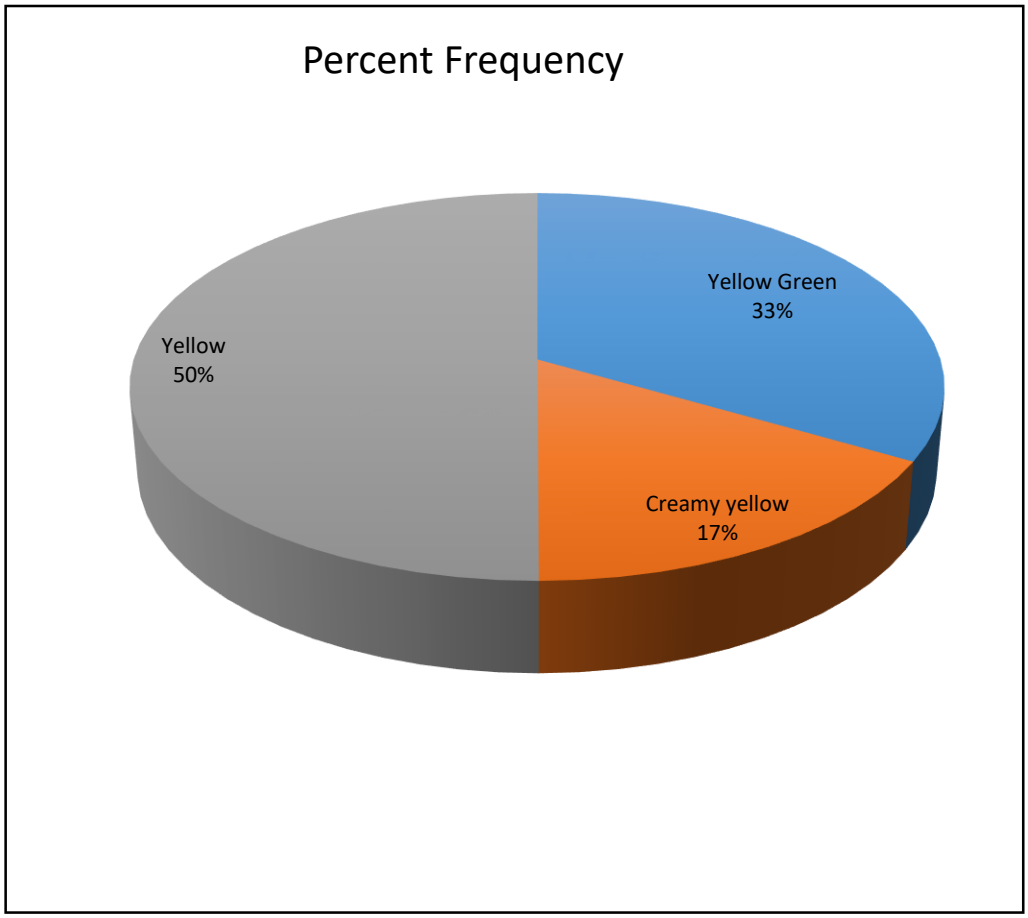

Figure 1. Frequency distribution (%) of fruit color among germplasms.

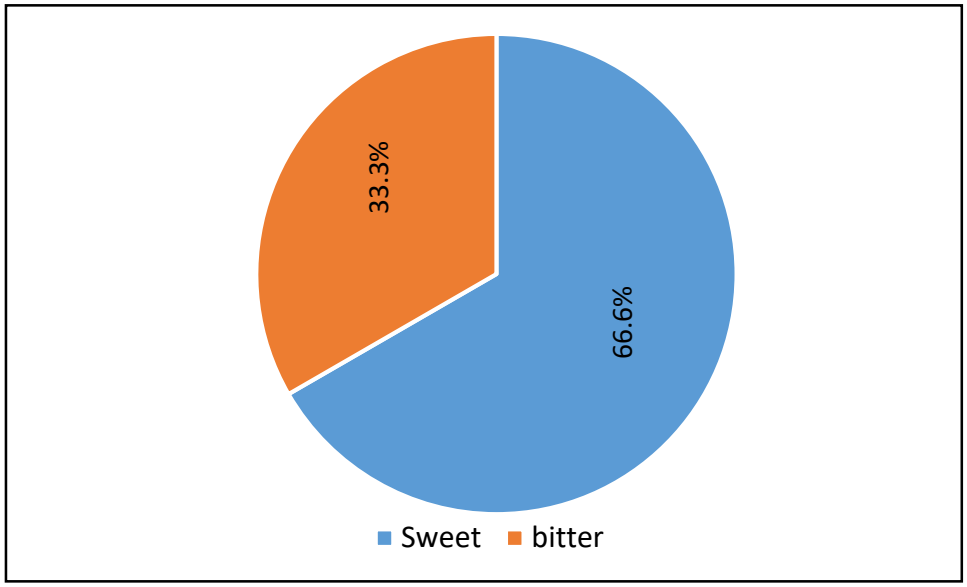

Figure 2. Frequency distribution (%) of kernel taste among the six Apricot germplasm

2.2.5 Total soluble solids (°Brix)

Total soluble solids were determined according to the association of official analytical chemists (AOAC, 2000) by using a hand refractometer at room temperature. So one drop of extracted juice from each sample was placed on refractometer prism and reading was recorded in the unit of brix.

2.3 Statistical Analysis

A frequency test, also known as a chi-square test, was used to statistically analyze the data using software Statistix (Version-10, Analytical Software). It compares the pattern of observed counts or frequencies to those that are anticipated to occur and Least Significant Difference (LSD) was performed for individual mean comparison at 5% probability level. The LSD determines the least significant difference between two means as if a test had been conducted on those two means alone (rather than on the means of all the groups combined). This enables you to compare two means from two different groups directly. Any difference that exceeds the LSD is regarded as a significant finding.

3. Result and Discussions

The research article discusses the results obtained from the data which is collected and then subjected to statistical analysis. We choose six different types of germplasms and are discussed one by one in detail both theoretically and graphically.

3.1 Fruit Colour

The frequency distribution of fruit colour among six different germplasm as shown in Figure 1. Considerable degree of variation was observed among fruit colour i.e. yellow color were more observed (50%), followed by yellow green (33%) and creamy yellow was observed with the lowest percentage of (17%). The germplasms Shernabi, Swat selection and Luizet showed yellow fruit color. However, the fruit colors of germplasms Portici and Vitilo were yellow green, while the germplasm Begali revealeds were creamy yellow color as shown in Table 1. These findings are in line with Asma, Kan et al. (2007).

3.2 Kernel Taste

The frequency distribution of kernel taste among germplasm as shown in Figure 2. Sweet taste was observed more frequently about 66.6% in different apricot varieties, while Bitter Taste were observed is about 33.3% in among germplasm. Our results highlighted that Begali, Shernabi, Swat Selection and Luizet were more sweet kernel taste germplasms. Whereas, the germplasms Protici and Vitilo were of bitter kernel taste shown in Table 1. The kernal taste is a genetically controlled trait which shows a genetic diversity among germplasms. Our results are more likely consistent with the findings of (Yilmaz, Paydas-Kargi et al. 2012).

3.3 Number of Fruits per kg

The frequency distribution of number of fruits per kg of six different germplasms as shown in Figure 3. The highest number of fruits per kg was recorded in the Begali germplasms (54), followed by Shernabi while the lowest number of fruits were observed in germplasm Luizet (17.33). The number of fruits per kg depends on the size of the fruits i.e., large size germplasm is less in number per kg, while small size of the germplasm is more in number. For example, Luizet germplasm produced fruits of larger size as compared to the remaining germplasms,

therefore the number of fruits per kg remained the least in this germplasm. Our results confirmed with the findings of (Amurrio, Varela Varela et al. 1995).

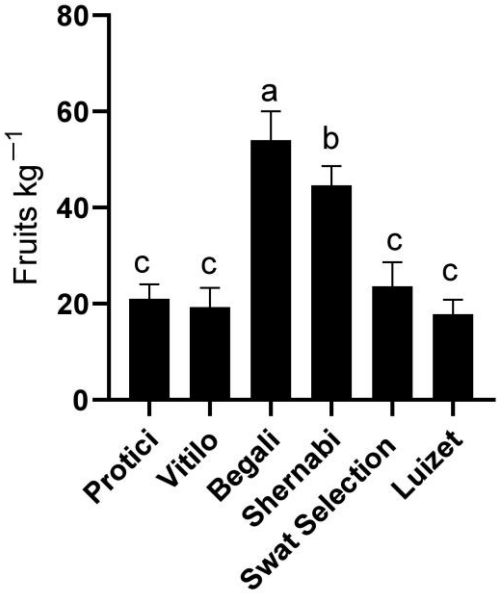

Figure 3. Number of fruits per kg of six apricot germplasms.

3.4 Nature of Stone

The frequency distribution for nature of stone among six different germplasms of Apricot are shown in Figure 4. Our results shown great variation in the nature of stone. The germplasm with free stone nature were more frequent (83%), while semi-cling stone nature was 17%. Further, the results showed that Protici, Vitilo, Begali, Shernabi and Swat Selection were free stone nature germplasm, whereas, the Luizet has semi-cling stone germplasm (Table 1). It is a genetic phenomenon whether a variety will have a free, semi cling or cling stone and result shows a great genetic variability. Our results are consistent with the findings of (Asma, Kan et al. 2007).

Table 1. Analysis of six different germplasms of Apricot for number of fruits/kg

Source	DF	SS	MS	F	P
Germplasms	5	3745.33	749.067	50.88	0.0001
Error	12	176.67	14.722		
Total	17	3922			
Grand Mean	**30.333**	**CV**	**12.65**		

Table 2. Analysis of six different germplasms of Apricot of total soluble solids.

Source	DF	SS	MS	F	P
Germplasms	5	53.667	10.733	7.67	0.0019
Error	12	16.793	1.3994		
Total	17	70.46			
Grand Mean	16.433	**CV**	7.2		

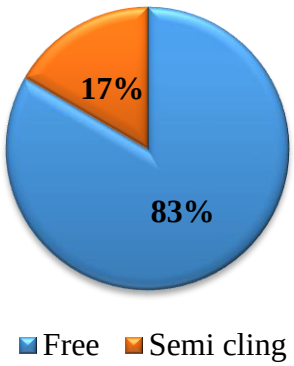

Figure 4. Frequency distribution (%) of stone nature among the germplasms.

3.5 Total Soluble Solids (ºBrix)

The frequency distribution of total soluble solids of six different germplasms of Apricot (Figure 5). The highest total soluble solids were measured in the germplasm Begali (18.067ºBrix). Whereas, Swat selection resulted in lowest total soluble solids (13.2ºBrix). In terms of total soluble solids,

the germplasm Begali is better and the Swat Selection is the worst. Our result is almost similar with Yilmaz, Paydas-Kargi et al. (2012).

Table 3. Qualitative traits of six apricot germplasms

Germplasms	Fruit color	Kernel Taste	Stone Nature
Protici	Yellow green	Bitter	Free
Vitilo	Yellow green	Bitter	Free
Begali	Creamy Yellow	Sweet	Free
Shernabi	Yellow	Sweet	Free
Swat Selection	Yellow	Sweet	Free
Luizet	Yellow	Sweet	Semi cling

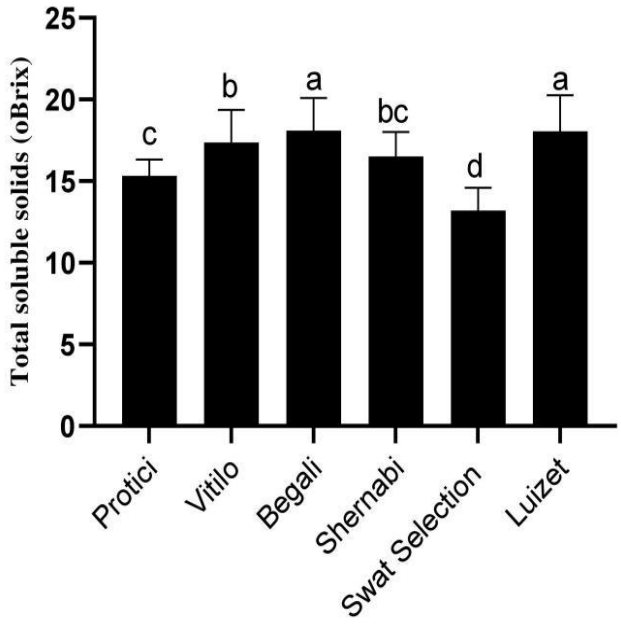

Figure 5. Total soluble solids (oBrix) of six apricot germplasms.

4. Conclusions

From the comprehensive quantitative and qualitative evaluation of the six different apricot varieties, it is concluded that this germplasm provides extensive genetic diversity in terms of production, color and taste of the fruits. Among the tested varieties, some varieties are best suitable for quantitative characters, whereas others were found suitable for qualitative characters. Hence, it is recommended that this germplasm may be used for breeding purposes in order to combine their good characters in a single variety suitable for cultivation in Pakistan. Also, recommended for young researchers to works on their ecology, taxonomy, pollen study and genetic diversity.

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Author's contribution:

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