Evaluation of the effect of biochar-based organic fertilizer on the growth performance of fennel and cumin plants for three years

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\textbf{ABSTRACT}

This three years field study examined the influence of application rates of manure from sheep and goat (S/G) and their mixture with wood-based or farm yard manure-based biochars (FYMB) on growth performance of \textit{Foeniculum vulgare} (fennel) and \textit{Cuminum cyminum} (cummin). The fertilizer amendment rates were 1.66, 3.32 and 6.64 t ha\textsuperscript{-1}, which were applied for three consecutive years in field. The nitrogen (N) and phosphorus (P), nitrogen use efficiency (NUE) and phosphorus use efficiency (PUE) in seeds and stover of test crops were analyzed for third year cropping only. Results demonstrated that in general, fertilizers did not influence yield of first and second year crops. The significant (P ≤ 0.05) positive influences of organic fertilizers were observed for third year crops and were of higher magnitude for \textit{C. cyminum} than \textit{F. vulgare} (126–306.6% increase for \textit{C. cyminum} and 24.5–48.4% increase for \textit{F. vulgare} than control). As compared to S/G applied at 6.64 t ha\textsuperscript{-1} rate, its co-amendment with wood-derived biochar at all application rates significantly reduced P in seeds; whereas, its co-amendment with both biochar types and at all application rates significantly reduced P in the stover of \textit{F. vulgare} (Table 3; P ≤ 0.05). For the crop \textit{C. cyminum}, there was no difference between treatments for the concentration of P in stover. The phosphorus use efficiency (PUE) of stover of \textit{F. vulgare} was significantly improved by 80–108% and by 60–79% in response to the application of S/G and its co-amendment with FYMB respectively than control. The PUE of seeds of \textit{F. vulgare} was increased by 100% than control in response to the co-amendment of manure with wood-derived biochar at high application rate (P ≤ 0.05). More profound significant improvement in PUE was observed for third year crop of \textit{C. cyminum}, as most of the treatments improved PUE of seeds by 171 – 561% and stover by 196–294% than control with no significant differences between fertilizer treatments. Results show no relationship between fertilizer application rates and life history trait of crops in space and time, since there was non-consistent and in general non-significant differences between fertilizer treatments for both crops.

1. Introduction

\textit{Foeniculum vulgare} and \textit{Cuminum cyminum} are two economically important medicinal crops. Both of these crops grow well in cool Mediterranean climate [1]. These two crops have high demand in Asia for the cure of gastrointestinal problems [2,3] and are used as condiments [4]. These crops are also recognized for possessing powerful immunomodulatory properties [5]. Furthermore, these herbs are found to have many biocidal components and recently published meta-analysis of Thiviya et al. [6] suggested that these herbs have essential oils that have antimicrobial and antibiofilm properties, which can be used as excellent alternatives of synthetic pesticides against soil-borne pathogens such as \textit{Chromobacterium violaceum}, \textit{Klebsiella pneumoniae} and \textit{Salmonella typhimurium}.

Cool Mediterranean climate is suitable for the growth of these crops [7,8]. Balochistan is the largest province of Pakistan, occupying approximately 44% of total land area of this country. More than 50% land area of this province has cool Mediterranean type climate [9], which is suitable for the growth of these crops. Currently, these crops are grown on test trial basis in various cold regions [7,8] and no documented evidence exists regarding cultivation of these crops on commercial basis in this province.

In Balochistan, use of manures as source of fertilizer in agricultural lands is a common practice. The farms of cow, buffalo and poultry are widely distributed in this province. However, manure from small ruminants (sheep/goat) is considered best as fertilizer over other manures from buffalo, cow and poultry. These small ruminants are part of private household farms and the production of
manure from these animals is less than manure from farm yards of cow and buffalo and poultry. The judicious use of this manure can play a significant role in the agriculture of this province. One way of maximizing the utility of this manure in agricultural lands is its mixture with pyrolyzed biocarbon (also known as biochar). Biochar is highly porous and recalcitrant to decomposition [10,11]. Due to these characteristics, it has a significant high adsorption capacity for nutrients; therefore, when it is applied in soil as a mixture with organic or synthetic fertilizers, it acts as nutrient-capture and a slow release fertilizer [11,12]. The mixture of biochar with fertilizers also improves other soil properties such as soil microbial functions, protects microbes from being grazed by other soil grazers and slows down the decomposition of soil organic matter by improving microbial carbon use efficiency and protecting soil organic matter from decomposition [11,13–15]. All these factors play a significant positive role in improving soil quality for crop growth.

Although these crops belong to the same family (Apiaceae), they possess different life-history traits. For instance, *F. vulgare* is perennial and approximately has three times greater biomass production than *C. cyminum*. Moreover, seeds of *F. vulgare* mature in late summer (August to September) while those of *C. cyminum* mature in mid-summer (June to July). As the *F. vulgare* and *C. cyminum* vary in biomass production the need of biochar-mixed manure from small ruminants as organic fertilizer may also vary in space and time. This research was conducted to evaluate the influence of such a biochar-based organic fertilizer on growth performance of these two crops over three years. Hypotheses of this research were 1) organic amendments improve plant growth performance 2) co-amendment of biochar with manure improves plant growth performance 3) *F. vulgare* requires fertilizer in high amount than *C. cyminum* 4) continuous low amendment of these fertilizers shows positive influence over time.

### 2. Materials and methods

#### 2.1. Study site

This research work was performed at Balochistan Agricultural Research and Development Centre (BARDC), Quetta, Pakistan (66° 57’ 20” E, 30° 11’ 39” N). The climate is Mediterranean, summer is dry and warm and winter is cold and snowfall also occurs. The total annual precipitation is less than 250 mm per year, but the area received unexpected heavy rainfall during third year of this experiment in 2019 (520.6 mm). The weather of study site during the three-years period of this research work is presented in Figure 1. Soil was silt loam, Brown Chernozem, had 450 g sand kg⁻¹, 50 g clay kg⁻¹, 500 g silt kg⁻¹, 9.9 g organic matter kg⁻¹ soil, electrical conductivity 0.25 dS m⁻¹, pH 7.9, 0.68, mg NO₃-N kg⁻¹ and 4.7 mg Olsen-P kg⁻¹.

#### 2.2. Manure and biochar

The manure from farm yard of buffalo and cow from the Quetta city was purchased. The air-dried manure was burned to black mass (biochar) in a domestic kiln (temperatures of domestic kilns are in the range of 350–550°C as reported by Mia et al. [16]). The slow-pyrolysis biochar from wood feedstock was obtained from timber market of Quetta city. This biochar is produced from the wood of *Acacia nilotica* L. in the underground kilns, which are known as ‘Bhatti’ in local language. The air-dried manure from goats and sheep was purchased from the market of small ruminants in Quetta city. The properties of biochars and small ruminant manure are presented in Table 1.

#### 2.3. Experimental design

The experiment was conducted in October 2017 and ended in August 2019. The crops (*F. vulgare* and *C. cyminum*) were cultivated separately and treatments were assigned randomly to the 1.5 × 1.5 m plots. There was approximately 0.25 m buffer between plots and there was no opening between plots to prevent horizontal flow of water (Supplementary Figure 1). The treatments were control (no fertilizer amendment), small ruminant (sheep or goat) manure (S/G) and mixture of wood-derived biochar or farmyard manure biochar (FYMB).

![Figure 1. Total monthly rainfall/precipitation (mm), mean monthly maximum and minimum temperatures (°C) during study period (2017 to 2019). The data was obtained from https://www.worldweatheronline.com/](image-url)
with S/G at 1:1 w/w ratio. Each fertilizer was applied at 0.25 kg plot⁻¹ (1.66 t ha⁻¹), 0.5 kg plot⁻¹ (3.32 t ha⁻¹) and 1 kg plot⁻¹ (6.64 t ha⁻¹) rates. In a Factorial experimental design, with two factors (fertilizer type and application rates) and each treatment was replicated three times. The biochars, manure and biochar-manure mixtures were amended in soil followed by their mixing in soil to 2–3 cm. The fertilizers of a given treatment were amended in the same plots for three consecutive years in autumn season (mid-October to early November). Treatments and their abbreviations are given in Table 2.

2.4. Sowing, harvesting and biomass estimation

The seeds were sown during the optimum time for their germination. The seeds of *F. officinales* were sown on 06, 06 and 5 March 2017, 2018 and 2019 respectively. The seeds of *C. cyminum* were sown on 12, 15 and 14 February 2017, 2018 and 2019 respectively. For both crops, 0.006 t ha⁻¹ seeds were broadcasted to plots (Supplementary Figure 1). The *F. vulgare* is perennial but was sown for three years also as plants were harvested with roots. The plots were irrigated once or twice (depending on need based on apparent dryness of soil and signs of leaf wilting) every week with groundwater. Plants were harvested at seed maturation stage. The harvest of *C. cyminum* was carried out in the first week of June (04, 06 and 07 of June in 2017, 2018 and 2019 respectively); whereas, *F. vulgare* was harvested after mid of August (17, 23 and 21 of August 2017, 2018 and 2019 respectively). Stover and seeds were oven-dried at 40°C for 48 hours followed by measurement of their biomass.

### 2.5. Nitrogen, phosphorus and nitrogen use efficiency and phosphorus use efficiency of seeds and stover

The oven-dried (dried at 40°C for 48 hours), homogeneously-grinded samples of seeds and stover of *F. vulgare* and *C. cyminum* of third year of cropping were analyzed for nitrogen (N), phosphorus (P), nitrogen use efficiency (NUE) and phosphorus use efficiency (PUE). The chemical analysis was carried out according to the wet oxidation method of Cottenie [17] and Jones [18] as described briefly by Manzoor et al. [19].

The NUE and PUE of stover and seeds as nutrient use efficiency ratio were determined by the formula of Baligar et al. [20] as:

\[
\text{NUE (or PUE) = \frac{\text{Biomass (or yield as t ha}^{-1}\text{)}}}{\text{Nitrogen or phosphorus in plant tissue}}
\]

### 2.6. Soil sampling, processing and analysis for total nitrogen and soluble mineral phosphorus

Soil samples were taken from 0–10 cm depth from each plot after harvest of third year crop (July 2019 for *C. cyminum* and August 2019 for *F. officinale*). Soil samples were collected in zip-lock bags, air-dried in laboratory and passed through 2 mm mesh sieve to remove debris and roots. Soil samples were extracted with 2 M KCl solution as 1:5 soil:KCl solution ratio [21]. The soil mineral nitrogen (N) as cumulative of nitrate (NO_3⁻) and ammonium (NH_4⁺), were analyzed by microtiter plate technique [22]. Soluble inorganic soil phosphorus (P) was analyzed with the method of D’Angelo et al. [23]. Instead of microplate reader, samples were analyzed for N and P with spectrophotometer (UV-1700 pharma spec, UV–visible, Shimadzu) [24].

### 2.7. Statistical analysis

Due to very large differences in the data sets of yield, NUE of stover biomass of *F. vulgare*, PUE of stover biomass and seeds of both crops and soluble inorganic P of soil, cultivated with both crops, non-parametric

### Table 1. Properties of biochar and manure used as soil amendments.

| Properties          | Wood biochar | FYM biochar | Small ruminant manure |
|---------------------|--------------|-------------|-----------------------|
| pH                  | 8.15         | 9.15        | 8.5                   |
| EC (ds m⁻¹)         | 14.6         | 8.55        | 13.2                  |
| Ash (g kg⁻¹)        | 45.0         | 293         | –                     |
| Nitrogen (g kg⁻¹)   | 4.3          | 2.9         | 12.8                  |
| Phosphorus (g kg⁻¹) | 0.3          | 5.6         | 8.3                   |

### Table 2. Application rates of biochar co-amended with NPK fertilizer and poultry manure on field plots cultivated with *Cuminum cyminum* and *Foeniculum officinale*. Treatments were applied to the same plot once a year for three years in the field experiment. Amendment rates are on dry weight basis.

| Treatment                  | Application rate (t ha⁻¹) of manure | Application rate (t ha⁻¹) of biochar |
|----------------------------|-------------------------------------|-------------------------------------|
| Control                    | 0                                   | 0                                   |
| S/G (manure from sheep and goat) | 1.66 t ha⁻¹ (21.3 kg ha⁻¹, 13.8 kg ha⁻¹ P₂O₅) | 0.83 t ha⁻¹ (3.57 kg ha⁻¹ N, 0.25 t ha⁻¹ P₂O₅) |
| S/G + wood-derived biochar | 3.32 t ha⁻¹ (42.4 kg ha⁻¹, 27.6 kg ha⁻¹ P₂O₅) | 0.83 t ha⁻¹ (3.57 kg ha⁻¹ N, 0.25 t ha⁻¹ P₂O₅) |
| S/G + FYMB                | 6.64 t ha⁻¹ (85.0 kg ha⁻¹, 55.1 kg ha⁻¹ P₂O₅) | 1.66 t ha⁻¹ (71.4 kg ha⁻¹ N, 0.49 kg ha⁻¹ P₂O₅) |
| S/G + FYMB                | 3.32 t ha⁻¹ (42.4 kg ha⁻¹, 27.6 kg ha⁻¹ P₂O₅) | 3.22 t ha⁻¹ (14.3 kg ha⁻¹ N, 0.99 kg ha⁻¹ P₂O₅) |
| S/G + FYMB                | 0.83 t ha⁻¹ (11.1 kg ha⁻¹, 6.9 kg ha⁻¹ P₂O₅) | 0.83 t ha⁻¹ (2.41 kg ha⁻¹ N, 4.65 t ha⁻¹ P₂O₅) |
| S/G + FYMB                | 1.66 t ha⁻¹ (21.3 kg ha⁻¹, 13.8 kg ha⁻¹ P₂O₅) | 1.66 t ha⁻¹ (4.81 kg ha⁻¹ N, 9.29 kg ha⁻¹ P₂O₅) |
| S/G + FYMB                | 3.32 t ha⁻¹ (42.4 kg ha⁻¹, 27.6 kg ha⁻¹ P₂O₅) | 3.32 t ha⁻¹ (9.63 kg ha⁻¹ N, 18.6 kg ha⁻¹ P₂O₅) |
Kruskal-Wallis Test was performed to measure significant differences between two treatment means. The data sets with significant difference were subsequently subjected to the pairwise comparison between treatment means of individual data sets, using Mann–Whitney test with Bonferroni correction. The raw data of all studied parameters are provided in supplementary files. The normally distributed data were analyzed with single factor ANOVA. The differences between treatment averages were measured with least significance difference test. All statistical analyses were carried out with CoSTAT C and Microsoft Excel.

3. Results

3.1. Yield and stover biomass

As compared to control treatment, application of fertilizers did not increase the yield and stover biomass for first and second year cropping of both crops; except that, application of S/G at high application rate (6.64 t ha\(^{-1}\)) significantly increased stover biomass of \textit{F. vulgare} than control and co-amendment of FYMB with S/G at 6.64 t ha\(^{-1}\) rate, significantly increased yield and stover biomass than control for \textit{C. cyminum} of first year crop (Figure 2; \(P \leq 0.05\)). For the third year cropping of \textit{F. vulgare}, S/G at 3.32, co-amendment of S/G with wood-derived biochar and with FYMB at 1.66 t ha\(^{-1}\) amendment rate significantly improved yield than control (Figure 2; \(P \leq 0.05\)). The \textit{F. vulgare} of third year cropping also had significantly higher stover biomass in response to the co-amendment of S/G with wood-derived biochar at 1.66 and 3.32 t ha\(^{-1}\) rate and with FYMB at 6.64 t ha\(^{-1}\) rate than control (Figure 2; \(P \leq 0.05\)). For \textit{C. cyminum} of third year cropping, all treatments increased yield and stover biomass than control except S/G at 1.66 t ha\(^{-1}\), co-application of S/G with wood-derived biochar at 3.32 (and 6.64 t ha\(^{-1}\) for yield only) and with FYMB at 6.64 t ha\(^{-1}\) treatments, which did not cause any change in yield than control (Figure 2; \(P \leq 0.05\)).

3.2. Nitrogen, phosphorus and NUE and PUE of stover and seeds

The amendment of S/G at 6.64 t ha\(^{-1}\) increased N and P in stover and its co-amendment with FYMB at 6.64 t ha\(^{-1}\) increased N in seeds of \textit{F. vulgare} as compared to control (Table 3; \(P \leq 0.05\)). As compared to S/G at 6.64 t ha\(^{-1}\) application rate, its co-amendment with wood-derived biochar at all application rates significantly reduced P in seeds; whereas, its co-amendment with both biochar types and at all application rates significantly reduced P in stover (Table 3; \(P \leq 0.05\)). For the crop \textit{C. cyminum}, there was no difference between treatments for the concentration of P in stover; whereas, co-amendment of S/G with wood-derived biochar at 1.66 t ha\(^{-1}\) had significantly increased P in seeds than its co-amendment with FYMB at the same application rate (Table 3; \(P \leq 0.05\)).

The application of S/G at 1.66 and 3.32 t ha\(^{-1}\) rates significantly improved the NUE and PUE of stover of \textit{F. vulgare} than control (Table 3; \(P \leq 0.05\)). Its co-amendment with FYMB at 3.32 and 6.64 t ha\(^{-1}\) increased PUE of stover of \textit{F. vulgare} than control treatment (Table 3; \(P \leq 0.05\)). The amendment of S/G at 1.6 t ha\(^{-1}\) and its co-amendment with wood-derived

![Figure 2](image-url)

**Figure 2.** Mean (± SD) of yield and stover biomass of \textit{F. vulgare} and \textit{C. cyminum} over three years. Bars with different letters indicate within year significant difference at \(P \leq 0.05\). Control; no fertilizer amendment, S/G; small ruminant manure, WB; wood-derived biochar, MB; manure-derived biochar, 1.6, 3.3 and 6.6 are amendment rates of fertilizers at 1.66, 3.34 and 6.64 t ha\(^{-1}\) rates respectively.
Table 3. Total N (mg g\(^{-1}\)), total P (mg g\(^{-1}\)), nitrogen use efficiency (NUE) and phosphorus use efficiency (PUE) of stover and seeds of *F. vulgare*, total P (mg g\(^{-1}\)) and PUE of stover and seeds of *C. cyminum* of third year crops.

| Treatment   | F. vulgare Seeds | F. vulgare Stover | C. cyminum Seeds | C. cyminum Stover | NUE F. vulgare Seeds | NUE F. vulgare Stover | PUE F. vulgare Seeds | PUE F. vulgare Stover | PUE C. cyminum Seeds | PUE C. cyminum Stover |
|-------------|------------------|-------------------|------------------|------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Control     | 42.6 ± 3.51\(\text{a}\) 37.3 ± 2.51\(\text{c}\) | 4.27 ± 0.17\(\text{d}\) 3.66 ± 0.31\(\text{b}\) | 3.82 ± 2.07\(\text{b}\) 2.71 ± 1.36 | 1.21 ± 0.2\(\text{a}\) 201 ± 6.2\(\text{b}\) | 12.1 ± 2.7\(\text{b}\) 203 ± 51\(\text{b}\) | 15.1 ± 2.0\(\text{c}\) 52.2 ± 21\(\text{b}\) |
| S/G 1.6     | 44.6 ± 3.05\(\text{b}\) 42 ± 5.29\(\text{d}\) | 3.60 ± 0.49\(\text{bcd}\) 3.70 ± 0.64\(\text{b}\) | 3.61 ± 2.41\(\text{b}\) 2.76 ± 0.91 | 1.63 ± 0.19\(\text{b}\) 36.9 ± 3.5\(\text{a}\) | 20.3 ± 1.8\(\text{b}\) 424 ± 88\(\text{a}\) | 62.6 ± 40\(\text{a}\) 206 ± 158\(\text{a}\) |
| S/G 3.3     | 413 ± 7.57\(\text{ab}\) 40.6 ± 2.08\(\text{bc}\) | 3.72 ± 0.77\(\text{bcd}\) 4.25 ± 0.64\(\text{b}\) | 3.00 ± 1.39\(\text{b}\) 3.45 ± 1.15 | 1.77 ± 0.73\(\text{bc}\) 37.1 ± 6.4\(\text{a}\) | 20.7 ± 11\(\text{a}\) 365 ± 109\(\text{a}\) | 42.0 ± 8.2\(\text{b}\) 108 ± 69.2\(\text{ab}\) |
| S/G 6.6     | 50 ± 5\(\text{a}\) 54.3 ± 18.6\(\text{ab}\) | 5.72 ± 1.95\(\text{a}\) 4.73 ± 0.86\(\text{a}\) | 2.61 ± 1.11\(\text{b}\) 3.99 ± 1.73 | 1.31 ± 0.58\(\text{a}\) 31.2 ± 20\(\text{b}\) | 13.0 ± 7.8\(\text{bc}\) 330 ± 185\(\text{b}\) | 41.0 ± 5.4\(\text{b}\) 94 ± 70\(\text{ab}\) |
| WB+S/G 1.6  | 50.6 ± 5.03\(\text{ab}\) 47 ± 12.1\(\text{bc}\) | 3.20 ± 0.25\(\text{f}\) 3.48 ± 0.09\(\text{b}\) | 3.37 ± 0.37\(\text{a}\) 3.44 ± 1.00 | 1.48 ± 0.27\(\text{c}\) 272 ± 13\(\text{b}\) | 23.8 ± 6.5\(\text{b}\) 365 ± 175\(\text{b}\) | 54.6 ± 63\(\text{a}\) 148 ± 149\(\text{ab}\) |
| WB+S/G 3.3  | 33.6 ± 3.51\(\text{b}\) 40 ± 3.46\(\text{bc}\) | 3.19 ± 0.36\(\text{d}\) 3.53 ± 0.31\(\text{b}\) | 2.83 ± 1.34\(\text{b}\) 3.94 ± 0.93 | 2.39 ± 0.47\(\text{a}\) 33.7 ± 9.2\(\text{a}\) | 25.1 ± 3.1\(\text{a}\) 396 ± 160\(\text{b}\) | 99.8 ± 70\(\text{a}\) 168 ± 92\(\text{ab}\) |
| WB+S/G 6.6  | 503 ± 6.02\(\text{ab}\) 40.6 ± 3.2\(\text{bc}\) | 4.03 ± 0.04\(\text{d}\) 3.50 ± 0.40\(\text{b}\) | 2.71 ± 0.72\(\text{b}\) 3.51 ± 1.87 | 2.03 ± 1.58\(\text{a}\) 514 ± 62\(\text{a}\) | 24.1 ± 16\(\text{a}\) 618 ± 76\(\text{a}\) | 65.5 ± 10\(\text{a}\) 168 ± 57\(\text{a}\) |
| MB+S/G 1.6  | 47.6 ± 8.73\(\text{a}\) 44 ± 10.5\(\text{bc}\) | 4.58 ± 2.23\(\text{bcd}\) 3.36 ± 0.26\(\text{b}\) | 1.80 ± 0.67\(\text{b}\) 3.46 ± 1.99 | 1.43 ± 0.34\(\text{b}\) 237 ± 3.6\(\text{b}\) | 16.7 ± 6\(\text{a}\) 317 ± 118\(\text{a}\) | 82.7 ± 53\(\text{a}\) 123 ± 111\(\text{a}\) |
| MB+S/G 3.3  | 416 ± 15.5\(\text{ab}\) 39.3 ± 6.02\(\text{bc}\) | 3.60 ± 0.86\(\text{bcd}\) 3.29 ± 0.44\(\text{a}\) | 2.72 ± 1.41\(\text{b}\) 2.34 ± 1.45 | 1.34 ± 0.60\(\text{bc}\) 289 ± 14\(\text{b}\) | 146 ± 5.1\(\text{a}\) 324 ± 79\(\text{a}\) | 63.7 ± 45\(\text{a}\) 192 ± 38\(\text{a}\) |
| MB+S/G 6.6  | 46 ± 4.35\(\text{a}\) 45 ± 2\(\text{a}\) | 3.76 ± 1.36\(\text{bcd}\) 3.44 ± 0.52\(\text{b}\) | 2.30 ± 1.19\(\text{b}\) 2.33 ± 0.92 | 0.16 ± 0.07\(\text{c}\) 269 ± 2.4\(\text{b}\) | 15.7 ± 6.9\(\text{a}\) 363 ± 103\(\text{a}\) | 58.9 ± 32\(\text{ab}\) 155 ± 122\(\text{a}\) |

Within column, values with different uppercase letters are significantly different at \(P \leq 0.05\).
biochar at 6.64 t ha\(^{-1}\) and with FYMB at 3.32 and 6.64 t ha\(^{-1}\) rates significantly improved PUE of stover than control (Table 3; \(P \leq 0.05\)). All fertilizer treatments (except co-amendment of S/G with wood-derived biochar at 1.66 t ha\(^{-1}\) rate) increased the PUE of seeds of \textit{C. cyminum} than control (Table 3; \(P \leq 0.05\)).

### 3.3. Soil total inorganic nitrogen and soluble mineral phosphorus

For the soil cultivated with \textit{F. vulgare}, as compared to control and S/G at 6.64 t ha\(^{-1}\) amendment rate, soil under treatments of S/G 1.66 t ha\(^{-1}\), co-amendment of S/G with wood-derived biochar at all application rates and its co-amendment with FYMB at 6.64 t ha\(^{-1}\) had significantly lower the concentration of total inorganic N (Figure 2; \(P \leq 0.05\)). Similarly, for the soil cultivated with \textit{C. cyminum}, as compared to control and S/G at 6.64 t ha\(^{-1}\) amendment rate, soil under treatments of S/G at 3.32 t ha\(^{-1}\), co-amendment of S/G with wood-derived biochar at 3.32 and 6.64 t ha\(^{-1}\) had significantly lower the concentration of total inorganic N (Figure 2; \(P \leq 0.05\)).

For \textit{F. vulgare}, soil co-amended with S/G and wood-derived biochar at 1.66 t ha\(^{-1}\) application rate, significantly reduced the concentration of soluble inorganic P as compared to control, soil co-amended with S/G and wood-derived biochar at 3.32 t ha\(^{-1}\) rate and co-amended with S/G and FYMB at 6.64 t ha\(^{-1}\) rate (Figure 3; \(P \leq 0.05\)). There was no difference between treatments for soluble inorganic P of soil cultivated with \textit{C. cyminum} (Figure 3). There was no difference in the concentration of total inorganic N of soil between the \textit{F. vulgare} and \textit{C. cyminum} cultivation; whereas, soluble inorganic P was significantly higher under cultivation of \textit{F. vulgare} than \textit{C. cyminum} (Figure 4; \(P \leq 0.05\)).

### 4. Discussion

#### 4.1. Yield and stover biomass production

For \textit{F. vulgare}; contrary to our hypothesis, with only one exception, as compared to control, amendment of S/G fertilizer and its co-amendment with biochars did not improve yield for first two cropping years. A significant increase than control was only observed for the stover biomass of first year cropping only, in response to the amendment of S/G fertilizer at higher application rate (6.64 t ha\(^{-1}\)). Interestingly, for \textit{C. cyminum} of first year cropping only, co-amendment of S/G with FYMB at 6.64 t ha\(^{-1}\) application rate, improved yield and stover biomass by 40 and 42% respectively than control. However, the results were not consistent for the second year cropping. Therefore, contrary to our hypotheses, neither S/G fertilizer as sole amendment nor its co-amendment with biochars had consistent positive influence on yield of both crops over first two years of cropping.

![Figure 3](image-url)

**Figure 3.** Mean (±SD) of total inorganic N and soluble inorganic P of soil samples. Bars with different letters indicate significant differences between treatments for a given crop (\textit{F. vulgare} or \textit{C. cyminum}) at \(P \leq 0.05\). Bars with * indicate difference between crops (\textit{F. vulgare} versus \textit{C. cyminum}) at \(P \leq 0.05\). Control; no fertilizer amendment, S/G; small ruminant manure, WB; wood-derived biochar, MB; farmyard manure-derived biochar, 1.6, 3.3 and 6.6 are amendment rates of fertilizers at 1.66, 3.34 and 6.64 t ha\(^{-1}\) rates respectively.
Our results are in agreement of previous reports, which revealed no influence of organic fertilizers and their co-amendments with biochars on yield of crops \cite{10,25,26}. For instance, a field study conducted at Banaras Hindu University, India with dry tropical climatic conditions, as compared to control treatment, Singh et al. \cite{27} found no difference in yield and stover biomass of wheat crop in response to the sole amendment of farmyard manure and its co-amendment of rice husk-derived biochar at 1:1 w/w ratio, applied at 5 t ha\(^{-1}\) rate. Our results are in contrast to other empirical evidences, which reported a significant positive response of crops of dry regions, to the amendment of organic fertilizers and their co-amendment with biochars \cite{28,29}. For instance, a field trial conducted in semi-arid climatic region, at the Agronomy Research Farm, University of Agriculture Peshawar, Arif et al. \cite{30} observed that the amendment of farm yard manure at 2.3, 3.5 and 4.7 t ha\(^{-1}\) rates and poultry manure at 2, 3 and 4 t ha\(^{-1}\) rates increased grain yield of maize by 11–34%. The co-amendment of these fertilizers with the wood-derived biochar, produced from Acacia tree as 10 t ha\(^{-1}\) amendment rate further increased yield by 7–16%.

Interestingly, significantly positive influences of these organic fertilizers on yield and stover biomass were seen for the crops of third year cropping. The significantly higher yield and/or stover biomass production of these crops in third year of cropping may be attributed to the two factors 1) continuous three years of amendment of fertilizers 2) unexpected heavy rainfall in the third year of cropping (520.6 mm in 2019 versus 90.2 mm and 34 mm in 2017 and 2018 respectively), which occurred during the growing season of these crops. The temperatures were also lower than usual during growing season of crops. Wet soil conditions under Mediterranean climate may reduce crop yield by reducing aeration to roots and causing diseases to crops \cite{31,32}. Organic amendments reduce bulk density and increase porosity of soil \cite{31}. Furthermore, biochar due to its dark color, keeps soil temperature warmer. For instance, soil amended with straw-derived slow-pyrolyzed biochar had 2°C higher temperature and difference between day and night temperatures was lower \cite{33}. These factors allow drainage of excess water and promote soil aeration, which in return helps crops cope with unexpected high rainfall-induced soil water saturation \cite{31}.

For third year of cropping, for \textit{F. vulgare}, amendment of S/G and its co-amendment with wood-derived biochar and FYMB at 3.32, 1.6 and 1.6 t ha\(^{-1}\) rate, increased yield by 23, 26 and 20% respectively. Likewise, co-amendment of S/G with wood-derived biochar at 1.66 and 3.32 t ha\(^{-1}\) rate and with FYMB at 6.64 t ha\(^{-1}\) rate significantly increased stover biomass of \textit{F. vulgare} by 50, 49 and 36% respectively. Interestingly, more dominant positive responses to the amendments of these fertilizers were observed for \textit{C. cyminum}. Out of nine fertilizer treatments, five treatments i.e. S/G at 3.32 and 6.64 t ha\(^{-1}\) rates, co-amendment of S/G with wood-derived biochar at 1.66 t ha\(^{-1}\) and its co-amendment with FYMB at 1.66 and 3.32 t ha\(^{-1}\) rates increased yield of \textit{C. cyminum} by 75, 67, 66, 56 and 57% respectively. The stover biomass of \textit{C. cyminum} also increased in response to the amendment of S/G at 3.32 and 6.64 t ha\(^{-1}\) rates, its co-amendment with wood-derived biochar at 1.66 and 6.64 t ha\(^{-1}\) rates and its co-amendment with FYMB at 1.66 and 3.32 t ha\(^{-1}\) by 82, 77, 75, 59, 56 and 69% respectively. The higher response of \textit{C. cyminum} to fertilizer amendments than \textit{F. vulgare} may be attributed to its approximately three times lower stover

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure4.png}
\caption{Mean (±SD) of total inorganic N and soluble inorganic P of soil samples under \textit{F. vulgare} and \textit{C. cyminum} cultivation. Bars with different letters indicate significant differences between soils cultivated with \textit{F. vulgare} and \textit{C. cyminum}.}
\end{figure}
biomass, which might reduce the high requirement of fertilizer for its growth. The other reason may be differential crop responses to fertilizer amendments. Published reports suggest that plant species vary in their interaction to their microbiome under a given environmental condition [34]. Therefore, type and application rate of fertilizer is not a stronger predictor of their influence on plant-soil microbe interaction and crop growth [34]. It merits further investigation to evaluate microbial abundance and community structure in the rhizosphere of these crops under various fertilizer types to evaluate if these fertilizers influence crop growth via modulating microbial functions in the rhizosphere.

Contrary to our hypotheses, we did not see a relationship of increase in yield or stover biomass of C. cymimum and F. vulgare with increasing the application rate of these fertilizers. Likewise, our results do not support our hypothesis that co-amendment of S/G with biochars further improve crop yield. Our results are in agreement to the findings of Zhang et al. [35], who did not find a linear increase in the yield of cotton with increasing the amount of poultry manure (applied at 3 and 6% of soil dry weight in field) or its mixture with cotton straw biochar (biochar applied as 1 and 3% of soil dry weight with manure at 3 and 6% of soil dry weight) under field conditions in arid region of Xinjiang Province, Northwest China.

4.2. Nitrogen, phosphorus and NUE and PUE of crops

Due to the fact that significant differences were only observed for third year cropping, seeds and stover biomass of only third year of cropping were considered for the quantification of NUE and PUE. Furthermore, due to limited time and funding, nitrogen in seeds and stover of C. cymimum was not measured.

The data for nitrogen, phosphorus and NUE and PUE revealed significant differences between fertilizer treatments and control as was observed for the yield and stover biomass production of third year of cropping. For instance, for F. vulgare, amendment of S/G at 6.64 t ha$^{-1}$ rate, increased N and P in stover biomass than control by 31 and 23% respectively, its co-amendment with FYMB at 6.64 t ha$^{-1}$ rate increased N in stover than control by 17%. These treatments however did not increase the NUE and PUE of stover and seeds of F. vulgare probably because these treatments did not increase yield or stover biomass than control. Interestingly, these treatments did not influence concentration of P and PUE of seeds and stover biomass of C. cymimum than control. It indicates that these crops had differential responses to the amendment rates of these organic fertilizers. Interesting findings can be seen for F. vulgare for the co-amendment of S/G with wood-derived biochar at all application rates as compared to when S/G was applied as sole fertilizer at 6.64 t ha$^{-1}$ rate. For instance, co-amendments of S/G with wood-derived biochar at all application rates significantly reduced the concentration of P in seeds by 30–44% and in stover by 25–26% when S/G was applied as sole fertilizer at 6.64 t ha$^{-1}$ rate. However, co-amendments of S/G with wood-derived biochar did not reduce the yield or stover biomass of F. vulgare than when S/G was applied as sole fertilizer at 6.64 t ha$^{-1}$ rate; therefore co-amendment of wood-derived biochar with S/G at 3.32 t ha$^{-1}$ rate significantly increase $≈2$ fold the PUE of seeds than S/G treatment at 6.64 t ha$^{-1}$ rate. This treatment (co-amendment of wood-derived biochar with S/G at 3.32 t ha$^{-1}$ rate) also increased the NUE of seeds of F. vulgare than control and when S/G was applied as sole fertilizer at 1.66 and 6.64 t ha$^{-1}$ rates. This indicates that the co-amendment of wood-derived biochar with S/G has the potential to increase the PUE of this crop.

As was observed for yield and stover biomass production of C. cymimum for the third year crop, significant influence of fertilizer amendments was also found on PUE of seeds and stover biomass of this crop as compared to control. Except for only one treatment of co-amendment of S/G with wood-derived biochar at lower application rates (1.66 t ha$^{-1}$), all other treatments increased the PUE of seeds of C. cymimum than control treatment by 63–85%. Likewise, application of S/G at 1.66 t ha$^{-1}$ and its co-amendment with wood-derived biochar at 6.64 t ha$^{-1}$ rate and with FYMB at 3.32 and 6.64 t ha$^{-1}$ rate significantly increased the PUE of stover of C. cymimum by 75, 69, 73 and 66% respectively than control. These treatments did not decrease the concentration of P in seeds and stover of this crop and most of these treatments increased yield and stover biomass of C. cymimum than control of third year cropping. This positive influence of these organic fertilizers on the yield and PUE of C. cymimum are promising in future for the farmers of this region to apply these fertilizers for the cultivation of C. cymimum. Furthermore, our results are well-aligned with previous reports, which suggest the positive influence of manure and its co-amendment with biochar on the improvement of NUE and PUE of crops under field conditions in arid and semi-arid regions [10,19,30].

4.3. Third year post harvest concentration of inorganic nitrogen and soluble inorganic phosphorus of soil

For the soil cultivated with F. vulgare, concentration of total inorganic N was significantly lower under treatment of co-amendment of S/G with wood-derived biochar at all application rates and its co-amendment with FYMB at 6.64 t ha$^{-1}$ rate than control treatment. Interestingly, these treatments (except co-amendment of S/G with wood-derived biochar at 6.64 t ha$^{-1}$) as
compared to control, increased yield of *F. vulgare* but did not influence concentration of N in seeds and stover than control. Likewise, similar results are found for soils cultivated with *C. cyminum*, in which as compared to control, soils under treatment of S/G at 3.32 t ha\(^{-1}\) and its co-amendment with wood-derived biochar at 3.32 and 6.64 t ha\(^{-1}\) had significantly lower the concentration of inorganic N. These treatments (except co-amendment of S/G with wood-derived biochar at 3.3 t ha\(^{-1}\) rate) also increased yield and/or stover biomass of *C. cyminum* than control treatment. The significant reduction in soil N but significant increase in yield and/or stover biomass in response to these organic fertilizer treatments may explain the reason of lower concentration of soil N. Our results are not in agreement to the published reports in this regard [30,36]. For instance, a pot-based study with maize crop, Naeem et al. [36] reported significantly higher yield, concentration of N and P in maize grain and higher concentration of soil N and P in response to the amendment of wheat straw-derived biochar, compost (made from fruit and vegetable wastes) and their co-amendment as 1% w/w in soil as compared to control treatment. The biochar and compost used in their study however had higher concentration of N and P than the fertilizers used in this study (14.2 and 18.8 mg kg\(^{-1}\) N in compost and biochar respectively and 0.3 and 2.49 mg kg\(^{-1}\) P in compost and biochar respectively; Naeem et al. [36]. The obvious reduction in soil N in response to the co-amendment of S/G with wood-derived biochar but not with FYMB (except for co-amendment of S/G with FYMB at 6.64 t ha\(^{-1}\) for *F. vulgare*-cultivated soil) may be due to the fact that wood-derived biochar had lower concentration of P than FYMB. Furthermore, biochar due to its porous nature, captures nutrients and act as slow-release fertilizer in soil [12,13,37,38]. Therefore, when it was co-amended with S/G, it might had captured nutrients and released them as per requirement of plants (and prevented their leaching); therefore, caused an increase in the yield and/or stover biomass of these crops instead of reduction in their growth.

Interestingly, the results for inorganic N in soil under various fertilizer treatments are not consistent with the results of soluble inorganic P. Except for only one treatment i.e. co-amendment of S/G with wood-derived biochar at lower application rate (i.e. 1.66 t ha\(^{-1}\)), which reduced the concentration of P in soil than control treatment under cultivation of *F. vulgare*, there was no difference between treatments. As explained above, this treatment caused an increase in the yield of this crop than control. This may explain reduction in the concentration of soluble inorganic P in soil.

Another finding can be seen that the concentration of P under cultivation of *F. vulgare* was almost three-fold significantly higher than the soil under cultivation of *C. cyminum*. But there was no difference in inorganic N in soil cultivated with *F. vulgare* versus *C. cyminum*. Interestingly, there was no difference in concentration of soil P between control treatments of both crops. The difference between *F. vulgare* versus *C. cyminum*-cultivated is seen for S/G at high application rate (3.32 t ha\(^{-1}\)) and its co-amendment with wood-derived biochar and FYMB at 3.32 and 6.64 t ha\(^{-1}\) rates respectively. Application of organic fertilizers and their co-amendment with biochar increase microbial activities in the rhizosphere of crops for P cycling (P mineralization and solubilization through secretions of organic acids) [39,40]. As *F. vulgare* has almost 3 times greater stover biomass than *C. cyminum*, this higher biomass may explain greater root biomass of this crop and ultimately higher P in soil than *C. cyminum*.

5. Conclusions

The amendments of S/G and its co-amendment with wood-derived biochar and FYMB at 1.66, 3.32 and 6.64 t ha\(^{-1}\) rates had no influence on yield and stover biomass production of both crops for first and second year of cropping. The continuous three years of amendment caused significantly improved growth performance of these crops for third year of cropping. As compared to S/G at higher application rate (6.64 t ha\(^{-1}\)), its co-amendment with wood-derived biochar reduced P in seeds and its co-amendment with both biochar types reduced P in stover. However, such results were not found for *C. cyminum*, co-amendment of S/G with wood-derived biochar at 1.66 t ha\(^{-1}\) had significantly increased P in seeds than its co-amendment with FYMB at the same application rate. This indicates differential responses of test crops to these fertilizers for the N and P uptake. Contrary to our hypotheses neither application rates of these fertilizers had differential influence on the yield of these crops over space and time nor co-amendment of S/G with biochars further improved their growth performance. However, *C. cyminum* exhibited more profound positive response to these fertilizers regarding yield and biomass production as well as PUE than *F. vulgare* only for the third cropping year. We recommend high amendment rates of these fertilizers than the rates that were used in this study to see a positive result for growth performance of these crops.

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Disclosure statement

No potential conflict of interest was reported by the author(s).
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