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Chili peppers photo by USDA, USA

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Smith J, Jones M Jr, Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

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Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

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Screening of rice germplasm for blast resistance in Nigeria

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Abstract. *Gabriel MG, Alhasan U, Mary Y, Munsur Y, Olufunmilayo A. 2022. Screening of rice germplasm for blast resistance in Nigeria. Asian J Agric 6: 1-6.* Rice blast (*Magnaporthe oryzae* T.T. Hebert) M.E. Barr) is an important destructive disease of rice that can lead to 80% yield loss. Germplasm responds differently to blast fungus. This study aimed to screen rice germplasm for blast resistance in Nigeria. The four genotypes, namely Institute for Research in Tropical Agriculture, France (IRAT) 109, JAMILA, Federal Agriculture Research Oryza (FARO) 52, and FARO 66, were evaluated in a completely randomized design with three replications in the screen house of the Department of Crop Protection, Institute for Agricultural Research Samaru, Nigeria. Data were collected on plant height, the number of plants infected with a blast, seedling vigor, tillering ability, blast disease score, and leaf blast estimated. Analysis of variance showed a highly significant difference ($P \leq 0.01$) for seedling vigor (0.03**) and disease index (17.24**), while significant ($P \leq 0.05$) variation was observed for a number of the leaf (3.79*). In contrast, there was no significant ($P > 0.05$) variation for plant height and tillering ability. The highest PCV (Phenotypic Coefficient of Variance) and GCV (Genotypic Coefficient of Variance), also broad-sense heritability, were observed in leaf blasts. IRAT 109 (0.6) depicted a high resistance, JAMILA moderately susceptible (Blast score 4.0), while FARO 52 (7.3) and FARO 66 (6.1) were susceptible. A significant difference among genotypes implies sufficient variation among the genotype screened, suggesting that progress can be made following selection.

Keywords: *Magnaporthe oryzae*, *Oryza sativa*, resistance, rice, screening

INTRODUCTION

Rice (*Oryza sativa* L.), with a genome size of 430 Mb (2n = 24), is the most widely consumed staple food for a large part of the world's human population (Amanullah et al. 2016; Perera and Dahanayake 2016). It may have originated in China and is now cultivated worldwide (Smith 2006). Global rice production is estimated to be 755.5.0 million tons per year, harvested from 162.1 million ha in more than 100 countries, with average productivity of 4.7 tons/ha (FAO 2019). In Africa, 14.2 million ha and 17.1 million ha of the land area were cultivated, with 33.2 million tons and 38.5 million tons harvested in 2018 and 2019, respectively, with similar productivity of 2.3 tons/ha. For example, Nigeria had rice production on a land area of 3.3 million ha and 5.3 million ha, with 6.8 million tons and 8.4 million tons harvested and a productivity of 2.03 tons/ha and 1.6 tons/ha in 2018 and 2019, respectively (FAO 2018; 2019).

Rice is an important staple food crop for more than half of the world's population, and it provides 27% of the calories in low and middle-income countries (Patil and Sharanagouda 2017; Susanto et al. 2017; Estiati 2019; Weerakoon and Somaratne 2020). Therefore, yield loss of rice production represents a significant threat to food security. Furthermore, it is stated that rice production must increase by 40% in 2030 to meet the ever-increasing demand (Khush et al. 2001). Hence population is increasing at an alarming rate, making food security a major challenge in the future.

Disease and pests are among the most important limiting factors that affect rice production. More than 70 diseases carried by fungi, bacteria, viruses, or nematodes have been reported on rice, and in severe cases, these losses could be up to 70-80% in some rice ecosystems (Deepak and Prasanta 2017). Among rice diseases, a blast is one of the most devastating worldwide (Fahad et al. 2019). Its wide destructiveness under conducive conditions results in yield loss ranging from 1-50% in various environmental conditions (Skamnioti and Gurr 2009). It is indicated that the genetic control of blast resistance is complex due to major and minor genes with complementary or additive effects and their environmental interactions (Zewdu et al. 2018).

Though chemical control has been successful, it adds to the cost of cultivation and contaminates the environment (Nalley et al. 2016). Deployment of host plant resistance genes is considered the best option for managing the disease, most of which are distributed in clusters (Raboin et al. 2016; Bano et al. 2017). The rapid changes in the virulence characteristics of the blast population raise a continuous threat to the effectiveness of existing blast-resistant varieties. Moreover, for the control of blast, breeding resistant varieties is an effective approach to reduce the use of pesticides and minimize rice losses due to this disease.

Continuous studies on blasts are important to overcome this disease and sustain rice production in the future.

Therefore, this study aimed to screen four genotypes of rice germplasm for blast resistance in Nigeria.

MATERIALS AND METHODS

Study site

The research was conducted in 2019 at the Institute for Agricultural Research (IAR) screen house, Ahmadu Bello University Zaria, Kaduna State, located in Samaru on 11°11'N, 7°38'E and 686 m above sea level in the Northern Guinea Savannah Ecological Zone of Nigeria. The area's average annual rainfall is about 1,058 mm, distributed within 160 days (Olanuga 1979).

Plant materials

The plant materials comprised of four rice genotypes obtained were Institute for Research in Tropical Agriculture, France (IRAT) 109, Federal Agriculture Research Oryza (FARO) 52, and FARO 66. In addition, those genotypes were obtained from African Rice (International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria), and JAMILA was obtained from the Zaria. The Rice blast resistance of each genotype is presented in Table 1.

Procedures

Fungal isolation

Plant samples were collected from a rice blast-infested field in Dogarawa, Bomo Village, and Samaru Kaduna State, Nigeria. Diseased leaves and nodes of rice panicles were placed on wet filter papers in a Petri dish for sporulation (Ou 1985).

Media preparation

Potato Dextrose Agar with streptomycin (PDAs) growth media was used. First, 200 g of sliced peeled potatoes were weighed in 1 l of water and boiled for 30 minutes. It was then filtered out, and 20 g of agar and 20 g of Dextrose were weighed into the solution and mixed well. The media was then autoclaved at 121°C for 15 minutes and left to cool to about 40 °C. It was then dispensed into sterile Petri dishes with a diameter of 9 cm (Ou 1985).

Leaf preparation

Infected leaf samples were cut into small portions, and sodium hypochlorite was added to them for three minutes and rinsed three times with distilled water (Ou 1985).

Culturing

Leaf samples were placed on the media in a petri-dish in the microflow chamber. Then the samples were taken to an incubator for observation of blast and viewed under a microscope (7-14 days) (Ou 1985).

Inoculation

The inoculum was harvested from the cultured plate and blended (mixed) in 200 mL of water, and the solution was sieved using a muslin cloth. River sand was sieved and sterilized using the oven. It was then used to create injury (rubbing) on the leaf's surface to aid in proper penetration of the inoculum. Next, the inoculum was sprayed on the surface of the plant leaf. And the residue was also used to inoculate the soil in the screen house. The inoculum's strength was determined using a hemocytometer (Gowrisri et al. 2019).

Spore storage and count

The cultured pathogen was subcultured into a McCartney bottle using a sterile picking pin. It was then kept for further use not to lose the pathogen (Gowrisri et al. 2019). The inoculum's strength was determined using a hemocytometer, and the spore count (50,000 spores/mL) was calculated (Smith et al. 1988).

Screening of genotype of rice

The four rice genotypes were screened for blast disease during the dry season of 2019 (February-March). The genotypes were raised in a completely randomized design with three replications in a 14 cm diameter wide and 12 cm deep pot. Five seeds of each of the materials were planted in 2 rows of 5 pots each. FARO 52 Plants were used as spreaders and inoculated with conidia harvested from the mycelia of a *Magnaporthe oryzae* (T.T. Hebert) M.E. Barr isolate. The test material (IRAT 109) surrounded six stands of susceptible rice cultivars as spreader rows. At the fourth-leaf stage (3-4 weeks after sowing), the seedlings were sprayed with spores of *M. oryzae* with about 30-40 mL of the spore suspension of the blast pathogen, and soil inoculation was done alongside the leaf inoculation. Water was sprayed 3-4 times a day to maintain high humidity. Inoculated seedlings were monitored to develop blast lesions (Gowda et al. 2015). The disease reaction of each genotype was recorded after 30 days of inoculation, following a standard 0-9 scale (SES IRRI 2013) (Table 2). In addition, data were collected on plant height (cm), seedling vigor (scale of 1-9), tillering ability (1-9), number of leaves affected, and leaf blast (1-9) as described by SES IRRI (2013).

Data analysis

The agronomical and physiological data collected were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of the Statistical Analysis System (SAS 2002). Fisher's protected least significant difference (LSD) test was used for comparison.

Randomized complete block design (RCBD) linear model: $y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$

Where,

y_{ijk} = Response of the experimental i^{th} treatment unit with the j^{th} replicate and k^{th} block.

μ = The overall mean
α_1= Effect of treatment
β_j = The Effect of block *j*
e_{ijk}= Random error
i = Number treatment unit;
j = Number of replication;
k = Number of block (Kittiwat et al. 2018).

Table 1. Description of four rice genotypes studied (Toungos 2016)

Characters	Genotype			
	IRAT 109	**FARO 52**	**FARO 66**	**JAMILA**
Rice blast resistance	Resistance	Highly susceptible	Susceptible to blast	Moderately Susceptible to blast
Medium maturity	Medium maturity between 90-100 days	Medium Maturity (100-110)	Medium Maturity (100-110)	Medium maturity between 90-100 days
Iron toxicity	Not resistant to toxicity	Resistant to Iron toxicity	Moderately tolerant to iron toxicity	Not resistant to iron toxicity
Yield	Medium Yielding	High Yielding	High Yielding	High-yielding landrace
Grain	Fat white grain	Moderately long white-grain	Medium, slender grains	Long White-grain
Submergence	-	Susceptible	Tolerant	-

Table 2. Description of the standard evaluation system scale for rice blast disease scoring (SES IRRI 2013)

Grade	Disease severity	Host response
0	Please say something	Highly Resistant
1	Small brown specks of pinpoint size	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin Lesions are mostly found on the lower leaves	Moderately resistant
3	Lesion type is the same as in 2, but a significant number of lesions on the upper leaves	Moderately resistant
4	Typical susceptible blast lesions, 3 mm or longer, infecting less than 4% of leaf area	Moderately Susceptible
5	Typical susceptible blast lesions of 3mm or longer, infecting 4 10% of the leaf area	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer, infecting 11-25% of the leaf area	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer, infecting 26-50% of the leaf area	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer, infecting 51-75% of the leaf area; many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible

Phenotypic and genotypic variability

The phenotypic and genotypic coefficient of variation were estimated according to Burton and Devane (1953) as follows:

$$\sigma_g^2 = \frac{MS_g - MS_e}{r}$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

Where:

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

σ_e^2 = error variance

MG_g = Mean squares of genotypes

MS_e = Mean square due to error

r = number of replications

$$PCV = \frac{\sqrt{\sigma^2 p}}{X} x100$$

$$GCV = \frac{\sqrt{\sigma^2 g}}{X} x100$$

Where:

PCV = Phenotypic coefficient of variation

GCV = Genotypic coefficient of variation

X = Grand mean value of the trait

Heritability

Heritability in a broad sense (h_b^2) for five characters was computed using the formula adopted by Allard (1960) as follows:

$$H^2 = \frac{\sigma^2 g}{\sigma^2 p} x100$$

Where:

h_b^2 = heritability in a broad sense

$\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

RESULTS AND DISCUSSION

Analysis of variance

The result of the analysis of variance (ANOVA) revealed highly significant ($P < 0.01$) variation for seedling vigor, number of infected leaves, and leaf blast. On the other hand, no significant ($P < 0.05$) difference was depicted by plant height and tillering ability (Table 3).

Estimated variance component

The result of the estimated variance component for genotypic variance range from 0.01 (seedling vigor) to 8.52 (leaf blast), while phenotypic variance range from 0.01 to 11.80 (plant height) (Table 4). The PCV value computed for the five traits ranged from 12.83 for seedling vigor to 65.24 for leaf blast, while GCV ranged from 5.93 tillering ability to 64.88 for leaf blast. The value of the phenotypic coefficient of variation was generally slightly higher than the corresponding genotypic coefficient of variation for all traits studied. High GCV was observed for leaf blast (64.88) and a number of the leaf (59.41), while moderate GCV was observed for seedling vigor (10.14). High PCV was observed for leaf blast (65.24) and a number of the leaf (61.21) while tillering ability (27.90), seedling vigor (12.83), and plant height (13.54) showed moderate PCV (Table 4).

Heritability in a broad sense

Broad sense heritability (Hb) estimates the total contribution of genetic variance to total phenotypic variance ranging from 4.52 tillering ability to 98.88 leaf blast. The heritability was high for leaf blast (98.88), the number of the leaf (94.20), and seedling vigor (62.50), which might be due to environmental influence on the expression of the traits. On the other hand, low heritability in the broad sense was observed for plant height (21.54) and tillering ability (4.52) (Table 4).

Mean performance of genotypes

Plant height has a mean of 25.38 and ranges from 23.88-29.25; seedling vigor has a mean of 0.90 and ranges from 0.8-1.0; tillering ability has a mean of 10.30 and ranges from 6.10-1 2.30, several leaves have a mean of 2.26 and ranged from 2.10-3.60 while leaf blast has a mean of 4.50 and ranged from 0.60-7.30 (Table 5). The significant difference for the traits studied at the 5% probability level was further confirmed by the mean comparison test using the respective LSD values. The mean performance indicated the different responses to the blast as there was variation.

Discussion

The average Nigerian rice productivity is still very low compared to other rice-producing countries worldwide. That is mainly due to insufficient improved rice varieties, disease, and other environmental factors affecting rice productivity. As a result, the present study screened four rice genotypes for resistance to rice blast and pattern of genetic variance present in the rice genotype. The presence of highly significant among the genotype for all characters except for tillering ability and plant height, which was non-significant, implies considerable variation among the genotype. Furthermore, the GCV values were relatively lesser than PCV for all traits. However, the difference between the PCV and GCV was relatively low for tillering ability, plant height, and vegetative vigor. That implies that the marked influence of environmental factors for the phenotypic expression of genotype was low; therefore, there is a higher chance of improving this trait through selection based on the phenotypic value of the traits. On the other hand, the difference in magnitude between the PCV and the GCV values was relatively high for the number of leaf and leaf blasts (Table 5) (Ikramullah et al. 2011).

Table 3. Mean squares of rice genotype screened for blast fungus

Source	Degree of freedom	Plant height	Seedling vigor	Tillering ability	Number of leaves affected	Leaf blast
Replications	1	1.98	0.01**	9.68	0.02	0.02^{ns}
Genotype	3	18.52	0.03**	16.52	3.79*	17.24**
Error	3	13.44	0.01	15.77	0.22	0.19^{ns}

Note: **: highly significance difference at (P> 0.01) probability level, *: significance difference at (P >0.05) probability level, ns: no significant

Table 4. Variance component of rice genotype for blast fungus

Traits	Variance component					
	σ_e^2	σ_g^2	σ_p^2	GCV %	PCV %	Hb
Plant height	13.44	2.54	11.80	6.28	13.54	21.54
Seedling vigor	0.01	0.01	0.01	10.14	12.83	62.50
Tillering ability	15.77	0.37	8.26	5.93	27.90	4.52
Number of leaves affected	0.22	1.79	1.90	59.41	61.21	94.20
Leaf blast	0.19	8.52	8.62	64.88	65.24	98.88

Table 5. Mean performance of rice genotype for blast fungus

Traits	Genotype	Mean	Range	CV%
Plant height	FARO 52	23.93	23.88-29.25	16.96
	FARO 66	23.88		
	IRAT 109	24.47		
	JAMILA	29.25		
	Mean	25.38		
	LSD	13.7		
Seedling vigor	FARO 52	0.80	0.8-1	0.01
	FARO 66	0.80		
	IRAT 109	1.00		
	JAMILA	1.00		
	Mean	0.90		
	LSD	0.00		
Tillering ability	FARO 52	6.10	6.10-12.30	38.56
	FARO 66	12.30		
	IRAT 109	12.00		
	JAMILA	10.80		
	Mean	10.30		
	LSD	12.64		
Number of leaves affected	FARO 52	3.60	2.10-3.60	20.85
	FARO 66	2.90		
	IRAT 109	2.40		
	JAMILA	2.10		
	Mean	2.26		
	LSD	1.49		
Leaf blast	FARO 52	7.30	0.60-7.30	9.77
	FARO 66	6.10		
	IRAT 109	0.60		
	JAMILA	4.00		
	Mean	4.50		
	LSD	1.40		

A high heritability estimate was observed for seedling vigor, leaf number, and leaf blast. The high estimated heritability value for these traits indicated that the variation observed was mainly under genetic control and was less influenced by the environment. Hence, the possibility of progress from a selection. That may be attributed to the uniform environment in the screen house (Muhder et al. 2020). Furthermore, high heritability was observed for leaf blast, leaves, and seedling vigor. It suggests that selection based on these characters would be effective for future crossing programs. This result agrees with Tuhina-Khatun et al. (2015) and Kamara et al. (2017), who also reported high broad-sense heritability in rice.

Observations recorded 30-40 days after sawing based on leaf blast severity following SES IRRI (2013) scale showed that IRAT 109 is highly resistant, and this was visible both in growth and vigor. The resistant ability of these genotypes may be genetics as it suppresses the organism's development causing these diseases. Although FARO 66 is susceptible, it was less susceptible when compared to FARO 52, which is the most susceptible among the four genotypes. The fungus was visible 40 days after sawing on the leaves, while JAMILA was moderately susceptible. This result agrees with Spyridon et al. (2009), who reported that varietal differences significantly contributed to the resistance or susceptibility of the rice to leaf blast and also in line with the work of Gbadeyan et al. (2018), who worked on screening of blast and genotype by environment interaction of rice. The different Response of the rice genotypes used in this study is important in selecting resistant varieties. These findings inspire further genetic studies to improve the genotypes through hybridization and selection programs.

In conclusion, the study highlighted rice germplasm with blast resistance in Nigeria and significant genetic variations for agronomically important traits, such as seedling vigor, leaf blast, and the number of leaves among the four rice genotypes that act as a pointer. Furthermore, the promising genotypes of IRAT 109 exhibited a significant level of resistance than JAMIIA, FARO 52, and FARO 66. Hence, IRAT 109 can be considered a candidate for a blast-resistant variety for possible progress.

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Production of quality seeds of chili using soil amendments

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Abstract. *Roy K, Khan AA, Rubayet MT, Haque MM. 2022. Production of quality seeds of chili using soil amendments. Asian J Agric 6: 7-14.* The present investigation was conducted to produce quality chili seeds using soil amendments. The soil was amended with vermicompost, colonized *Trichoderma*, mustard oil cake, cow dung, and poultry manure for quality seed production. The fruit yield (3.63 t ha^{-1}) and seed yield (2.40 t ha^{-1}) were higher from colonized *Trichoderma* amended plot. All the organic amendments, such as colonized *Trichoderma*, vermicompost, poultry manure, mustard oil cake, and cow dung, increased the yield of chili seeds compared to the control. Quality characteristics of harvested seeds, such as germination, vigor, and 1,000 seed weight, were found higher in colonized *Trichoderma* amendment plots. Five fungi, namely *Aspergillus* sp., *Fusarium* sp., *Colletotrichum capsici* (Syd. & P.Syd.) E.J.Butler & Bisby, *Curvularia* sp., and *Alternaria alternata* (Fr.) Keissl. were found to be associated with seeds, and the infection rate of different fungi varied from 1.70 to 9.20%. The lowest (9.80%) total seed-borne infection was also recorded in seeds of colonized *Trichoderma* amended plot. The highest (3.73) benefit-cost ratio was obtained from colonized *Trichoderma*, followed by poultry manure (3.39) and vermicompost (3.33) amendment. Thus, soil amendment with colonized *Trichoderma*, vermicompost, or poultry manure is suggested to produce quality chili seeds with a higher yield.

Keywords: Cow dung, mustard oil cake, poultry manure, seed health, *Trichoderma*, vermicompost

INTRODUCTION

Chili is an important spice of the world and is extensively grown in Bangladesh. It belongs to the genus *Capsicum* and the family Solanaceae and is a source of vitamins A, E, and C. Color and flavor extract from chili are used in both food and industries. Some varieties are grown all over Bangladesh in Rabi and Kharif seasons. In 2018-19, the area was 43,947 and 195,256 acres, and the production was 43,452 and 10,602 tons in Kharif and Rabi seasons, respectively (BBS 2020). Quality seed is an essential component of crop production. Farmers mainly depend on their seeds of inferior quality. Poor seed quality is an important factor among the various factors responsible for the low yield of the crop. The application of chemical fertilizers may increase its yield. But the imbalanced and excessive use of chemical fertilizers degrades the soil and the environment (Higa 1991; Lestari et al. 2017; Jaikhisun et al. 2018; Purba et al. 2020). The organic amendments used with water, such as traditional thermophilic composts, have long been used as an effective method to improve soil structure, increase soil fertility, microbial diversity, populations, and activity, improve soil moisture-holding capacity, and increase crop yields (Zink and Allen 1998). Vermicomposting is the best method to recycle solid waste towards achieving sustainable solid waste management. During the passage of organic substrate through the gut, earthworms convert the nutrients from organic matter into bioavailable forms. Various enzymes and hormones are mixed with the digested material, stimulating plant growth and protecting plants from pathogen infestation (Gajalakshmi and Abbasi 2004). *Trichoderma* is a genus of fungus strains that live as symbionts on plant roots and have qualities that encourage plant growth and development (Harman et al. 2004). *Trichoderma* species have long been recognized as bio-agent for controlling plant disease and increasing yield by increasing phytohormones production, such as jasmonic and salicylic acid (Silva et al. 2019). *Trichoderma* as a biological agent may be a cost-effective and efficient technique. Many studies on *Trichoderma* are underway, focusing on their ability to alleviate abiotic stress; nevertheless, the specific knowledge of mechanisms related to their ability to modulate diverse plant abiotic stress factors is still lacking. The commercialization of *Trichoderma* biofertilizer has encouraged farmers. *Trichoderma* is used in all crops, with or without additives; however, when used as an amendment with compost, *Trichoderma* biofertilizer may produce greater results than any other fertilizer. The poultry industry is one of the world's largest and fastest-growing agricultural enterprises. The majority of the litter made by the poultry business is currently used as a source of nutrients and soil amendment on agricultural land (Bolan et al. 2010). It has been applied to surrounding crops and pasturelands to recycle nutrients, primarily nitrogen (N), phosphorus (P), and potash (K) (Lorimor and Xin 1999).

Cow dung is important organic manure. It is also called soil life and is important in sustainable soil fertility and crop productivity. The biggest benefit of cycling and recycling organic matter in soils is the overall soil

environment improvement and supply of nutrients, especially N, P, K, and S. Moreover, it provides nutrients and improves soil's physical and chemical properties like porosity and water-holding capacity. In addition, cow dung is a harbor of rich microbial diversity, containing different species of bacteria (*Bacillus* sp., *Corynebacterium* sp., and *Lactobacillus* sp.), protozoa, and yeast (*Saccharomyces* and *Candida*) (Nene and Thapliyal 2002). About 60% of the seed mustard oil cake is generated as a by-product during oil extraction. It is a rich source of nitrogen (4.8%), potassium (as K_2O-1.3%), and phosphorous (as P_2O_5-2%), which are essential requirements to maintain the fertility of the soil and the proper growth of a plant. Organic manure provides several benefits by minimizing production costs and is an environment-friendly cultivation method. Consumers have been increasingly expecting high-quality, safe food and are particularly interested in organic items (Ouda and Mahadeen, 2008). Inorganic fertilizer is made of synthetic materials; when an excess of the application occurs, the soil becomes toxic. However, organic crop additives are becoming more popular as people become more aware of the harmful impacts of inorganic fertilizers on crop productivity and growing environmental and ecological concerns. Considering the above facts, the study aimed to know the effect of various soil amendments on the quality of chili seed production.

MATERIALS AND METHODS

Location of the experimental field

The experiment was conducted from November 2018 to May 2019 at Bangabandhu Sheikh Mujibur Rahman Agricultural University's Plant Pathology field in Gazipur, Bangladesh. The experimental site was located at 24°09 N latitude and 90°26 E longitudes, with an elevation of 8.4 meters above sea level, and it belongs to the Salna series, which represents the sallow Red Brown Terrace soil that falls under the order of inceptisols and the Madhupur Tract's Agro-Ecological Zone (AEZ No. 28).

The climate of the experimental area

In the experimental area, the minimum and maximum air temperature varied between 11 to 27°C and 14 to 34°C, soil temperature in 10 cm, 20 cm, and 30 cm depth varied between 16 to 27.5°C, 16.5 to 28°C, and 17 to 28.5°C, respectively, groundwater table varied between 15.60 m and 18.56 m and the total amount of rainfall was 2.92 mm during the entire cropping period.

The soil of the experimental field

The soil of the study site was silty clay loam to clay loam in texture and belonged to the Madhupur tract's AEZ No. 28, with a pH of 5.8 to 6.5 and an ECE of 25.28 (Haider 1991). The organic carbon and organic matter of the soil sample collected from the experimental area were 0.75 and 1.12, respectively, determined with the help of the Soil Science Department of BSMRAU before applying soil amendments.

Materials collection

Chandra Mukhi chili seeds of Lal Teer, mustard oil cake, and chemical fertilizers were collected from a retailer at Joydebpur in Gazipur. Vermicompost was collected from Pajulia Village at Gazipur. *Trichoderma* culture was taken from the Plant Pathology division of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, and poultry manure and cow dung were gathered from the BSMRAU's nearby farm. The chickpea meal substrate was prepared and inoculated with *Trichoderma harzianum* Rifai. The *T. harzianum* was allowed to colonize in a chickpea meal for 21 days (Arefin et al. 2019). After 21 days, the colonized chickpea meal substrate was air-dried and stored in a conical flask a 10°C (Liton et al. 2019; Das et al. 2019). The mustard oil cake was soaked in water for 24 hours and kept in a hole to adjust the soil temperature (Rubayet and Bhuiyan 2016). After three days, the mustard oil cake was ready to incorporate into the soil.

Raising of seedlings

Seedlings of chilies were cultivated in a seedbed. The seedbed was (1.2 m × 3 m) in size. The soil was well prepared and turned into a loose friable, and dry mass using good tilth. All weeds and stubbles were removed, and the soil was amended with well-decomposed cow manure. On 1 November 2018, ten-gram seeds were sown and then covered with light dirt. Within six days of sowing, the seedlings emerged. Weeding and irrigation were performed as needed. Plants were ready for transplanting after 35 days of seed sowing.

Design and layout of the experiment

The experiment was set up in a three-replication randomized complete block design (RCBD). The total area was divided into three equal blocks. Each block consisted of six plots, where six treatments were allotted randomly. There were 18 unit plots in the experiment. The area of a unit plot was 4 m^2, which contained 16 plants at a spacing of 40 cm × 40 cm. The distance between two blocks and two plots was kept at 1.00 m and 0.50 m, respectively.

Land preparation

The soil was thoroughly prepared for agricultural production, and tilth was ensured. The trial field land was ploughed with a motorized tiller and then laddered to achieve the desired tilth. Larger clods were broken into smaller pieces, and the land corners were spaded. After ploughing and laddering, all the stubbles and uprooted weeds were removed, and the land was ready. The field layout and design of the experiment were followed after land preparation.

Application of fertilizers

According to Fertilizer Recommended Guide Bangladesh Agricultural Research Council (BARC), a generalized dose of 1.60 kg TSP, 1.2 kg MoP, and 0.78 kg (half dose) urea fertilizer were given in all units plots at the rate of 223 kg ha^{-1}, 167 kg ha^{-1} and 218 kg ha^{-1}. The rest of the half dose (0.78 kg) of urea fertilizer was applied after 30 days of transplanting.

Treatments of the experiment

The experiment had five treatments for soil amendment. However, the control was maintained where no soil amendment was included. Thus, the experiment had six treatments altogether. In each 4 m^2 plot, 2.00 kg vermicompost (@ 5 t ha^{-1}), 0. 27 kg colonized *Trichoderma* (@ 0.90 t ha^{-1}), 0.80 kg mustard oil cake (@ 2 t ha^{-1}), 4.00 kg cow dung (@ 10 t ha^{-1}) and 2.00 kg poultry manure (@ 5 t ha^{-1}) were applied during final land preparation in the respective plot as per the layout of the experiment.

Transplanting of seedlings

Healthy and uniform 35 days old seedlings were uprooted from the seedbed and were transplanted in the experimental plots on 6 December 2018.

Intercultural operations

After transplanting the seedlings, different intercultural operations were accomplished for better growth and development of the plants. Weeding was accomplished whenever necessary to keep the crop free from weeds. Irrigations were given throughout the growing period by garden pipes and watering canes. The first irrigation was given immediately after the transplantation, while the second was done per requirement.

Harvesting

Fruits were harvested at 6 to 7 days intervals during an early riping stage when they attained marketable size. Harvesting was started on 15 March 2019 and continued until 25 May 2019.

Data collection

Five plants were randomly selected from each plot for data collection to prevent the border effect and achieve the best level of precision. Data on the following parameters were collected from the sample plants during the experiment.

Plant height

The plant height of the selected five plants was measured in cm from the base of the plant to the terminal growth point of the main stem at 65 days of planting up to observe the growth of plants, and the average height was computed.

Number of branches per plant

The number of branches per plant was counted 65 days after transplanting (DAP) from tagged plants. The average of five plants was computed and expressed in an average number of branches per plant.

Number of fruits per plant

The total number of fruits produced in a plant was counted and recorded.

The dry weight of fruits

After harvesting, the fruits were dried in the sunshine. The moisture content was maintained at 9.00%. A digital weighing balance was used to measure the fruit weight of each plot.

The dry weight of seeds

The exocarp was cut with a sharp blade, and the chili seeds were removed from the fruit. A digital weighing balance was used to measure the seed weight of each plot.

Disease incidence

After transplanting the seedlings, data on various diseases like *Cercospora* leaf spot, *Fusarium* wilted plants, and anthracnose of fruits was recorded in each field plot. Then, the percentage of disease incidence was calculated following the formula given below:

$$\% \text{ disease incidence} = \frac{\text{No. of infected leaves/plants/fruits}}{\text{Total no. leaves/plants/fruits investigated}} \times 100$$

The infected samples of leaves, plants, and fruits were collected in the laboratory, and associated pathogens were isolated and identified.

Quality of harvested seeds

Germination capacity

Germination capacity was determined from four hundred seeds (4×100) into replicates of 100 seeds of each sample sown in blotting paper in a petri dish. After seven days of sowing, results were recorded as a percentage of the number of seeds germinated on the Petri dish. The average percentage was recorded to the nearest whole number. The germination percentage was computed using the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds tested}} \times 100$$

Seed vigor index

The rate of germination is measured vigor index (VI). First, normal germinated seeds were removed and counted daily. That was started on the first seeding and continued up to 14 days. Then, an index was computed for each replication by dividing the number of germinated seeds removed each day by the day they were removed (Sen and Ghosh 1999). Seed VI can be computed by the following formula (AOSA 1983):

$$\text{Vigour index} = \frac{\text{No. of germinated seeds in first count}}{\text{No. of days to first count}} + \ldots + \frac{\text{No. of germinated seeds in last count}}{\text{No. of days to last count}}$$

Electrical conductivity

Four replications of 50 seeds of each sample were weighted (to the nearest mg) and then soaked in 50 mL de-ionized water for 24 hours at 25±1°C. After 24 hours, the water of the beaker containing seeds was decanted to separate the seeds. The electrical conductivity of the decanted water containing seed leachate was then measured with a conductivity meter (Model-CM-30ET). The electrical conductivity (EC) was expressed in µS/cm.

Thousand seed weight

Thousand seed weight was calculated for each seed sample. First, the seed sample was divided into three sub-samples, and ten replicates of 100 seeds from each sub-sample were counted at random. Thus, the weight of replicates (l00×l0×3 replicates) was added together, and the resulting mean weight was recorded at the 1,000 seeds weight of the seed sample (ISTA 2006).

Seed health test

Regarding fungi associated with chili seeds, seed health was tested following International Rules for Seed Health Testing (ISTA 1976). A sub-sample of 400 seeds was randomly selected from each sample. Seeds were plated on sterilized and moist blotter paper in a 9 cm Petri dish. Twenty-five seeds were plated in each Petri dish, maintaining equal distances from seed to seed. After plating, the seeds were incubated at 25±2°C temperature. The filter paper was kept moist by adding sterilized water whenever necessary. Data on the prevalence of seed-borne fungi grown on the planted seeds were recorded after seven days of incubation. Fungi associated with the seeds were observed under a binocular microscope. Based on the morphological characters, fungi were identified using appropriate keys (Mathur and Kongsdal 2003). When fungus identification was impossible by observing the growth characteristics under a stereo-microscope, temporary mounts were prepared and examined under a compound microscope for detailed morphology. Seeds exhibiting different fungi were expressed in percentage based on the planted seeds.

Statistical analysis

All data were subjected to statistical analysis by analysis of variance (ANOVA). Microsoft Excel and Statistics 10 software programs were used wherever appropriate, and means were compared according to the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Effect of soil amendment on chili seed production

Plant height

The result showed that significantly higher plant height was found in colonized *Trichoderma* amended plot (79.70 cm), followed by vermicompost (75.10 cm), poultry manure (74.6 cm), mustard oil cake (73.00 cm) and cow dung (71.40 cm) amended plots. There were no significant differences among vermicompost, poultry manure, and mustard oil cake amended plots in the context of plant height. The lowest (66.80 cm) plant height was found in the control (Table 1).

Number of branches/plant

A significant maximum number of branches per plant was found in colonized *Trichoderma* treated plot (17.00), followed by vermicompost (15.00), poultry manure (14.00), mustard oil cake (14.00), and cow dung (13.00) amended plots. There were no significant differences in the number of branches/plants in vermicompost, poultry manure, and mustard oil cake-treated plots. The minimum (10.00) number of branches was found in the control treatment (Table 1).

Number of fruits/plant

The maximum number of fruits per plant was found in colonized *Trichoderma* applied plot (171.00), followed by vermicompost (161.00), poultry manure (149.00), mustard oil cake (144.00), and cow dung (140.00) applied plots. There was no significant difference in some fruits per plant in colonized *Trichoderma* and vermicompost applied plots. The minimum (120.00) number of fruits per plant was found in the control (Table 1).

Fruits and seed yield (t ha^{-1})

The highest fruit yield was recorded in the colonized *Trichoderma* applied plot (3.63 t ha^{-1}), which was statistically similar to the vermicompost applied plot (3.53 t ha^{-1}). The yield of chili was statistically similar with vermicompost (3.53 t ha^{-1}), poultry manure (3.50 t ha^{-1}), and mustard oil cake (3.35 t ha^{-1}) applied plot. The lowest (2.08 t ha^{-1}) yield was found in the control. The highest seed yield was recorded in colonized *Trichoderma* applied plot (2.40 t ha^{-1}), followed by the vermicompost applied plot (2.18 t ha^{-1}). The seed yield was statistically similar with vermicompost (2.18 t ha-1), and poultry manure (2.12 t ha^{-1}) applied plots. The lowest (1.35 t ha^{-1}) seed yield was found in the control (Table 1).

Table 1. Effect of different soil amendments on growth and yield parameters of chili

Soil amendments	Height of plants (cm)	Number of branches/plants	Number of fruits/plants	Weight of fruits (t ha^{-1})	Weight of seeds (t ha^{-1})
Control	66.80d	10.00d	120.00d	2.08d	1.35 e
Vermicompost	75.10b	15.00b	161.00ab	3.53ab	2.18b
Colonized *Trichoderma*	79.70a	17.00a	171.00a	3.63a	2.40a
Mustard oil cake	73.00bc	14.00bc	144.00c	3.35b	2.00c
Cow dung	71.40c	13.00c	140.00c	2.45c	1.55d
Poultry manure	74.60b	14.00b	149.00bc	3.50ab	2.12b
CV (%)	2.29	5.44	6.21	4.06	4.78

Note: Means followed by the same letter (s) in a column are not significantly different at a 5% level

Percentage of increase in fruit and seed yield of chili

Results revealed that all the treatments under soil amendments increased the fruit and seed yield significantly compared to the control, but their efficacy was not similar. The highest fruit yield was increased in colonized *Trichoderma* (74.00%) treated plots, followed by vermicompost (69.00%), poultry manure (68.00%), and mustard oil cake (61.00%) treated plots. The cow dung-treated plot recorded the lowest (17.00%) fruit yield increase (Figure 1). The highest seed yield increase was recorded in colonized *Trichoderma* (77.00%) treated plots, followed by vermicompost (61.00%), poultry manure (57.00%), and mustard oil cake (48.00%) treated plots. The lowest (15.00%) seed yield increase was recorded in the cow dung-treated plot (Figure 2). Molla et al. (2012) reported an above 200% yield increase was recorded by BioF/Compost (household/ kitchen/wastes composted by *Trichoderma*) over the control, but a 30.40% yield increase was recorded by BioF/Compost over the standard dose of N:P:K. Rahaman et al. (2012) experimented with an 18% yield increase recorded by cow dung over a standard dose of N:P:K in field studies of chili. Hassan et al. (2013) showed that a 35.00% yield was increased by poultry manure as soil amendment over the standard dose of N:P:K. The present experimental results agreed with the previous findings of various researchers (Molla et al. 2012; Rahman et al. 2012; Simi et al. 2019) as the soil amendment increases the yield of chili fruits and seeds.

Disease incidence

Three diseases, *Cercospora* leaf spot, *Fusarium* wilt, and anthracnose of fruits, were recorded in the experimental chili field. The causal organism of diseases identified *Cercospora capsici* Heald & F.A.Wolf, *Fusarium oxysporum* Schltdl. and *Colletotrichum capsici* (Syd. & P.Syd.) E.J. Butler & Bisby, respectively (Table 2).

Incidence of *Cercospora* leaf spot

The highest *Cercospora* leaf spot was recorded in the control plot (10.83%), followed by cow dung (8.41%), mustard oil cake (6.83%), and poultry manure treated plot (4.50%). The lowest incidence of *Cercospora* leaf spot was recorded in colonized *Trichoderma* (3.00%) treated plot, followed by the vermicompost (4.42%) treated plot (Table 2).

Incidence of wilt-infected plants

The highest *Fusarium* infected plants were observed in the control plot (18.75%), followed by cow dung (10.40%), poultry manure (8.30%), mustard oil cake (6.25%), and vermicompost (6.25%) treated plot. The disease incidence was statistically similar in vermicompost, poultry manure, and mustard oil cake-treated plot. The lowest (2.10%) *Fusarium*-infected plant was observed in colonized *Trichoderma*-treated plot (Table 2).

Incidence of anthracnose in chili fruits

The highest anthracnose-infected fruits were recorded in the control plot (11.41%), followed by cow dung (7.91%), mustard oil cake (6.91%), poultry manure, and vermicompost (5.16%) applied plot. The lowest anthracnose was recorded in colonized *Trichoderma* (2.91%) applied plot, followed by the vermicompost (5.16%) applied plot (Table 2).

Quality of harvested seeds under soil amendments

Germination percentage

The highest germination was recorded in seeds collected from *Trichoderma* amended plot (77.00%), followed by vermicompost (70.00%). The germination percentage of harvested seeds collected from the soil-amended plot with poultry manure, mustard oil cake, and cow dung was 69.00%, 67.00%, and 65.00%, respectively. The germination percentage of harvested seeds collected from vermicompost and poultry manure amended plot was statistically similar. The lowest germination percentage was recorded in the seeds of the control plot (63.00%), which was statistically similar to the cow dung (65.00%) amended plot (Table 3).

Vigor index (VI)

Vigor indices of seeds collected from six treatments ranged from 28.50-40.50. The highest VI was recorded in colonized *Trichoderma* (40.50) amended plot, followed by vermicompost (37.58), poultry manure (35.40), mustard oil cake (33.84), and cow dung (29.40) amended plots. But VI of seeds harvested from poultry manure and mustard oil cake amended plots was statistically similar. The control plot recorded harvested seeds' lowest (28.50) VI (Table 3).

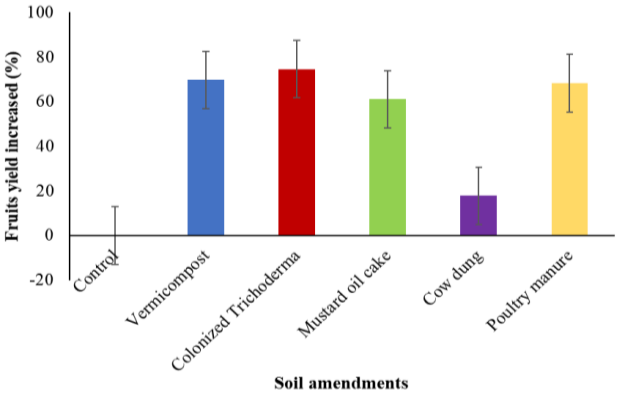

Figure 1. Increased percentage of fruit yield under different soil amendments over the control

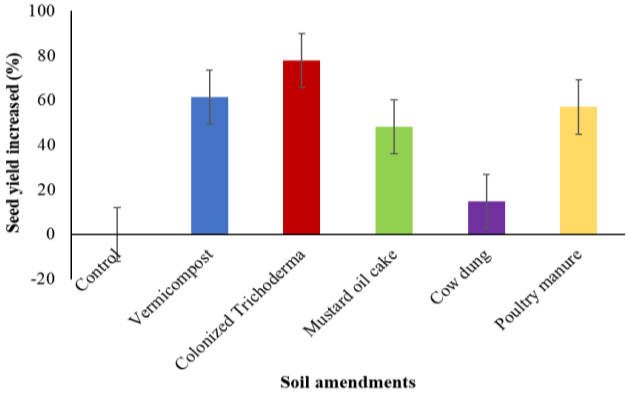

Figure 2. Increased percentage of seed yield under different soil amendments over the control

Electrical conductivity

The electrical conductivity of seeds harvested from soil amended field varied from 0.11 to 0.18 µS cm^{-1}. The lowest electrical conductivity was recorded in colonized *Trichoderma* treated plot (0.11 µS cm^{-1}), followed by vermicompost (0.13 µS cm^{-1}), poultry manure (0.14 µS cm^{-1}), mustard oil cake (0.14 µS cm^{-1}) and cow dung (0.15 µS cm^{-1}) treated plots. Regarding electrical conductivity, there was no significant difference among vermicompost, poultry manure, and mustard oil cake-treated plots. The electrical conductivity of seeds harvested from poultry manure, mustard oil cake, and cow dung-treated plots was also statistically similar. The maximum (0.18 µS cm^{-1}) electrical conductivity was recorded in the control (Table 3).

Thousand seed weight

The thousand seed weights of harvested seeds collected from soil-amended fields ranged from 3.90 to 4.80 g. The highest thousand seed weight was obtained from colonized *Trichoderma* amended plot (4.80 g), followed by vermicompost (4.60 g), poultry manure (4.53 g), mustard oil cake (4.40 g) and cow dung (4.10 g) amended plot. The thousand seed weights of vermicompost and poultry manure amended plots were statistically similar. The lowest (3.90 g) thousand seed weight was recorded in the control plot (Table 3).

Prevalence of seed-borne fungi of harvested seeds

Total seed-borne fungal infection in harvested chili seeds ranged from 9.80 to 39.70%. The highest infection was recorded in the control plot (39.70%), followed by cow dung (30.10%), mustard oil cake (23.50%), poultry manure (17.75%), and vermicompost (16.60%) amended plot. The lowest (9.80%) fungal infection was recorded in seeds of colonized *Trichoderma* amended plot. The identified fungal species were *Aspergillus* sp., *Fusarium* sp., *Curvularia* sp., *Co. capsici*, and *Alternaria alternata* (Fr.) Keissl. (Table 4). The most predominant fungus was *Aspergillus* sp., ranging between 4.50 to 9.00%. The highest *Aspergillus* sp. was found in control (9.00%), followed by the amended plot of cow dung (7.50%). The prevalence of *Aspergillus* sp. in seeds was statistically similar in cow dung (7.50%) and mustard oil cake (6.30%) amended plots and vermicompost (3.90%) and poultry manure (4.50%) amended plot. The lowest (2.00%) *Aspergillus* sp. infection was recorded in colonized *Trichoderma* amended plot (Table 4). The second most fungi recorded in seeds was *Fusarium* sp. ranging from 2.10 to 9.20%. The highest *Fusarium* sp. infection was recorded in seeds of the control plot (9.20%), followed by cow dung (6.10%), mustard oil cake (4.90%), vermicompost (3.90%), poultry manure (3.30%) amended plot. The lowest (2.10%) fungal infection was recorded in colonized *Trichoderma* amended plot (Table 5). The highest incidence of *Co. capsici* was recorded in seeds harvested from the control plot (7.70%), followed by cow dung (5.80%), mustard oil cake (4.20%), poultry manure (3.10%), vermicompost (2.80%), amended plot. Among them, mustard oil cake (4.20%), poultry manure (3.10%), and vermicompost amended plot (2.80%) were statistically similar. The lowest incidence of Co. capsici was recorded in seeds of colonized *Trichoderma* (1.70%) amended plot, which was statistically similar to the vermicompost (2.80%) amended plot. Curvularia sp.'s prevalence in seeds varied from 1.90-6.80%. The highest incidence of *Curvularia* sp. was found in the control plot (6.80%), followed by cow dung (4.80%), mustard oil cake (4.00%), and poultry manure (3.25%) amended plot. The lowest (1.90%) incidence of *Curvularia* sp. was recorded in seeds of colonized *Trichoderma* amended plot. The prevalence of *Alternaria* varied from 2.10 to 7.00%. The highest *A. alternata* infection was noted in seeds of the control plot (7.00%), followed by cow dung (5.90%), mustard oil cake (3.90%), poultry manure (3.60%), vermicompost (3.10%) amended plot. Among them, vermicompost and colonized *Trichoderma* amended plots were statistically similar. The lowest (2.10%) *Alternaria* infection was recorded in seeds of colonized *Trichoderma* amended plot (Table 4).

Table 2. Percentage of different disease incidences of chili in the field under soil amendments

Soil amendments	*Cercospora*-affected leaves (%)	*Fusarium* wilted plants (%)	Anthracnose-affected fruits (%)
Control	10.83a	18.75a	11.41a
Vermicompost	4.42d	6.25b	5.16d
Colonized *Trichoderma*	3.00e	2.10c	2.91e
Mustard oil cake	6.83c	6.25bc	6.91c
Cow dung	8.41b	10.4b	7.91b
Poultry manure	4.50d	8.30bc	5.25d
CV (%)	7.07	48.33	8.71

Note: Means followed by the same letter (s) in a column are not significantly different at a 5% level

Table 3. Effect of different soil amendments on quality parameters of harvested chili seeds

Soil amendments	Germination (%)	Vigor index	Electrical conductivity (µS cm^{-1})	Thousand seed weight (g)
Control	63.00d	28.50e	0.18a	3.90e
Vermicompost	70.00b	37.58b	0.13c	4.60b
Colonized *Trichoderma*	77.00a	40.50a	0.11d	4.80a
Mustard oil cake	67.00c	33.65c	0.14bc	4.40c
Cow dung	65.00d	29.40d	0.15b	4.10d
Poultry manure	69.00bc	35.40c	0.14bc	4.53b
CV (%)	1.45	3.71	7.0	1.33

Note: Means followed by the same letter (s) in a column are not significantly different at a 5% level

Table 4. Incidence of fungi associated with seeds produced by soil amendments

Soil amendments	Seed-borne fungi (%)					
	***Aspergillus* sp.**	***Colletotrichum capsici***	***Fusarium* sp.**	***Curvularia* sp.**	***Alternaria alternata***	**Total**
Control	9.00a	7.70a	9.20a	6.80a	7.00a	39.70
Vermicompost	3.90c	2.80cd	3.90d	2.60e	3.10d	16.30
Colonized *Trichoderma*	2.00d	1.70d	2.10e	1.90f	2.10e	9.80
Mustard oil cake	6.30b	4.20c	4.90c	4.00c	3.90c	23.30
Cow dung	7.50b	5.80b	6.10b	4.80b	5.90b	30.10
Poultry manure	4.50c	3.10c	3.30d	3.25d	3.60cd	17.75
CV (%)	12.32	17.23	9.49	8.41	10.78	

Note: Means followed by the same letter (s) in a column are not significantly different at a 5% level

Percentage of reduction in total seed-borne fungi of chili

Results of soil amendments with six treatments, namely vermicompost, colonized *Trichoderma*, poultry manure, mustard oil cake, and cow dung against seed-borne fungi of chili, are presented in Figure 3. All the soil amendments significantly reduced the percent seed-borne infection of total fungi (*Aspergillus* sp., *Co. capsici*, *Fusarium* sp., *Curvularia* sp., and *A. alternata*) compared to the control plot. The reduction percentage of fungi varied from 24.00 to 75.00%. The highest reduction percentage was obtained by colonized *Trichoderma* amendment (75.00%), followed by vermicompost (59.00%), poultry manure (55.00%), mustard oil cake (41.00%), and cow dung amendment (24.00%) (Figure 3). The experiment results suggested that organic soil amendments and application of colonized *Trichoderma* in soil reduced disease incidence of chili in the field and reduced the seed-borne pathogens in harvested seeds. Different researchers also reported that organic soil amendments and colonized *Trichoderma* reduce disease incidence (Kapoor 1996; Punja et al. 2002; Prasad et al. 2002; Kashem et al. 2005; Hassan et al. 2013; Sultan and Guffar 2013; Tapwal et al. 2015). Sultana and Ghaffar (2013) reported that maximum increase in plant size (20.00 cm), seed germination (86.00%), and up to 2.20% reduction in seedling mortality caused by *Fusarium* sp. in cucumber by mustard oil cake in the field study.

Kapoor (1996) found groundnut and mustard oilcake at a 2.00 % concentration of soil (w/w) was most effective in reducing pathogen population (>70%) and disease incidence. Hassan et al. (2013) reported that 63.30% anthracnose and 5.00% *Cercospora* leaf spot reduction were obtained using poultry manure as a soil amendment. Punja et al. (2002) evaluated greenhouse waste, windrow dairy solids, vermicompost dairy solids, and commercially available biological control agents to reduce disease incidences of *Fusarium* root and stem rot caused by *F. oxysporum* f.sp. *radicis-cucumerinum*, and *Pythium* damping-off and crown rot, caused by *Pythium aphanidermatum* (Edson) Fitzp. They reported that all three composts reduced root and stem rots to some degree, and autoclaved compost lost its suppression, suggesting the microbial antagonism involved. Prasad et al. (2002) found that soil treated with *T. harzianum* showed 61.50% disease control in chickpeas, while Kashem et al. (2005) observed about 30.00% disease control in lentil seeds. Tapwal et al. (2015) found that *T. harzianum* recorded maximum growth inhibition (34.20%) against *A. alternata*, followed by *F. oxysporum* (27.04%), *Cochliobolus lunatus* R.R.Nelson & F.A.Haasis (25.64%), *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (15.00%) and minimum for *Rhizoctonia solani* J.G.Kühn (5.10%). Bhat et al. (2016) reported that *T. harzianum* inhibited mycelia growth by 40.00 to 50.00% in vitro and increased yield up to 30.00% in the Haveri District, while at Bellary, *T. harzianum* reduced wilt incidence by 39.70%.

Benefit-cost ratio

The benefit-cost ratio obtained from different soil amendments, including control, varied from 1.98 to 3.73. The highest (3.73) benefit-cost ratio was obtained from colonized *Trichoderma* amendment soil, followed by poultry manure (3.39), vermicompost (3.33), mustard oil cake (2.83), and cow dung (2.31) amendment and the lowest (1.98) benefit-cost ratio was obtained from control (Table 5).

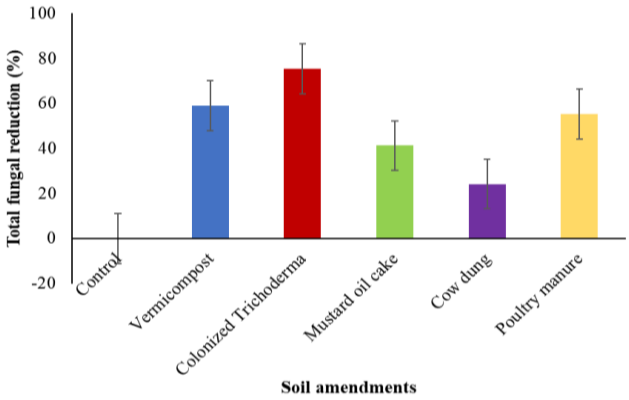

Figure 3. Percentage of reduction of total seed-borne fungi under different soil amendments over the control

Table 5. The benefit-cost ratio of soil amendments and the control

Soil amendments	Cost (Tk. ha^{-1})	Selling price (Tk. ha^{-1})	Benefit-cost ratio
Control	136561	270000	1.98
Vermicompost	166561	555000	3.33
Colonized *Trichoderma*	160861	600000	3.73
Mustard oil cake	176561	500000	2.83
Cow dung	156561	362500	2.31
Poultry manure	156561	530000	3.39

In conclusion, growth characteristics like plant height, the number of branches, and fruit and seed yield were the highest in the colonized *Trichoderma* amended plot, followed by the vermicompost amended plot. The disease incidence of *Cercospora* leaf spot, *Fusarium* wilt, and anthracnose of fruits was minimum in colonized *Trichoderma* amended plot. The highest seed yield was increased by colonized *Trichoderma* soil amendment (77.00%), followed by vermicompost (61.00%) and poultry manure (57.00%) amendment. The quality of harvested seeds was better in colonized *Trichoderma* and vermicompost amended plots.

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Influence of climate change on agricultural sustainability in India: A state-wise panel data analysis

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Abstract. *Singh AK, Kumar S, Jyoti B. 2022. Influence of climate change on agricultural sustainability in India: A State-wise panel data analysis. Asian J Agric 6: 15-27.* This study developed Economic Efficiency Index (EEI), Social Equity Index (SEI), and Ecological Security Index (ESI) as an assessment of the Agricultural Sustainability Index (ASI) in 17 Indian states during 1990-2017. The Composite Z-Score method integrated 32 economic, social, and ecological security factors to create ASI, EEI, SEI, and ESI. Subsequently, it examined the impact of climatic factors on ASI using linear, log-linear, and non-linear regression models through state-wise panel data during the said period. The descriptive results indicate that agricultural sustainability was positively associated with economic efficiency, social equity, and ecological security. Therefore, factors related to economic efficiency, social equity, and ecological security would help improve sustainability in the Indian agricultural sector. Furthermore, there was high diversity in economic efficiency, social equity, and ecological security across the Indian state. The ratio of agriculture's Gross Domestic Product (GDP) and gross irrigated area with the gross sown area, landholding size, yield of food-grain and oilseed crops, and cropped area under food-grain crops were observed to be the most influencing factors of economic efficiency. The total literacy rate, female literacy rate, and rural literate population were the most crucial factors in improving social equity. Ecological security was improved with increased forest area, pastureland, and cropping intensity. Furthermore, the empirical results also showed that maximum temperature had a negative influence; and economic efficiency, social equity, and ecological security positively influenced agricultural sustainability in India. Therefore, India needs to take effective climate policy action to mitigate the negative impact of climate change in the agricultural sector and its allied activities to increase sustainable agricultural development in India. Subsequently, this study provided several policy suggestions to reduce climate change risk in the Indian agricultural sector.

Keywords: Agricultural sustainability, climate change, ecological security, economic efficiency, India, social equity

Abbreviations: ASI: Agricultural Sustainability Index; CMIE: Centre for Monitoring Indian Economy; CDR: Credit Deposit Ratio; ESI: Ecological Security Index; ESI: Ecological Security Index; EDI: Economic Development Index; EEI: Economic Efficiency Index; EnSI: Environmental Sustainability Index; GoI: Government of India; GHGs: Greenhouse Gases; GDP: Gross Domestic Product; IMD: Indian Metrological Department; OECD: Organization for Economic Co-operation and Development; RBI: Reserve Bank of India; SEI: Social Equity Index; VIF: Variance Inflation Factor

INTRODUCTION

The agricultural sector is the sole sector to meet the food demand of people, provide raw materials to industries, create employment for agricultural laborers, and gives fodder to livestock. Moreover, at present agriculture sector is facing several challenges due to overwhelming population growth, industrialization, urbanization, scarcity of ecosystem services, decreasing size of landholding, rising cost of cultivation, shifting of farmers towards the non-agricultural sector, low agricultural R&D expenditure, insignificant support from Government and climate change at a global level (Singh and Hiremath 2010; Latruffe et al. 2016; Kareemulla et al. 2017; Kumar et al. 2017; Bakari et al. 2018; Singh and Issac 2018; Lampridi et al. 2019; Mili and Martínez-Vega 2019). Furthermore, the world's population is expected to reach 11.2 million by 2100 (Lampridi et al. 2019). Thus, the agriculture sector will be vulnerable due to the activities above in the future. Hence, there is an urgent to implement conducive policies to increase agricultural sustainability worldwide.

The notion of sustainability of the agriculture sector is that it meets the food security of people and can maintain the farmers' profitability, provide fodder to all livestock in the long-term, and tolerate the negative impact of soil degradation, socio-economic demand, and gradually degrading environment (Hensen 1996). Also, it includes farming methods that do not negatively affect the environment and the economic accessibility of farmers (Rostami and Mohammadi 2017). Moreover, it maintains economic viability and social welfare by sustaining the quality of natural resources (Hensen 1996). Finally, it also integrates the environment, economic efficiency, and social equity to increase food production (Gaetano 2010; Fallah-Alipour et al. 2018). Existing researchers have defined agricultural sustainability and used its indicators per their views. For instance, Gomez et al. (1996) and Hensen (1996) have argued that the agricultural system can be

sustainable when it can meet the farmer's need for productivity, profitability, stability, and social equity and preserves the quality of natural resources.

Agricultural sustainability is a situation in which a firm efficiently produces enough food for people without damaging the ecosystem services (Asadi et al. 2013). Agricultural sustainability may be defined as efficient and optimum food-grain and non-food-grain crops that do not negatively impact ecosystem services and human health (Kareemulla et al. 2017). Fallah-Alipour et al. (2018) defined agricultural sustainability as protecting the environment and improving agricultural production and human well-being. Furthermore, several studies have claimed that agricultural sustainability includes socio-economic and bio-ecological dimensions (De Koeijer et al. 2002; Sharma and Shardendu 2011; Talukder et al. 2020). Factors associated with the environment, social and economic development are also the determinants of agricultural sustainability (Latruffe et al. 2016; Ryan et al. 2016; Lampridi et al. 2019; Mili and Martínez-Vega 2019). Therefore, agricultural sustainability can be achieved by maintaining economic, social, and environmental development. Valizadeh and Hayati (2021) claimed that social equity, human well-being, stability, productivity, and efficiency of resources are the determinants of agricultural sustainability. Although, agricultural production activities have several negative impacts on ecosystem services (i.e., land, water, forests, air, soil-erosion, and biodiversity), contributing to around 31% of greenhouse gas globally (Talukder et al. 2020). Thus, achieving sustainability in the agricultural sector would be challenging to maintain environmental, economic, and social development in larger agrarian economies like India and China (Zhen and Routray 2003).

In India, a large segment of society is engaged in the agricultural sector (Ghabru et al. 2017). Therefore, India must increase agricultural sustainability to meet people's food security and provide raw materials to agro-based industries. Also, India is going to be the most populated country by 2025. Thus, there would be a requirement for more food to feed the growing population in India. Several studies have assessed the influence of various activities on the agricultural sector in India using primary and secondary data at the district, state, region, and country levels. Most studies have examined the impact of climatic and non-climatic factors on agricultural production and productivity in India (e.g., Kumar et al. 2016, 2017). However, in India, limited studies could measure agricultural sustainability across states (except, Kareemulla et al. 2017). Few studies could assess the association of climatic factors with agricultural sustainability in India. Also, previous studies could not address the climate change impacts on agricultural sustainability in India. Due to highlighted research gap, this study addressed the following research objectives: To develop the Agricultural Sustainability Index (ASI), Economic Efficiency Index (EEI), Social Equity Index (SEI), and Ecological Security Index (ESI) in Indian states for some time of 1990-2017. To examine the influence of climatic factors on estimated ASI using state-wise panel data in India.

MATERIALS AND METHODS

Study area and sources of data

For this study, 17 states of India were considered with time series of 28 years (i.e., 1990-2017). The following Indian states were considered from various regions: (i) Southern Region: Andhra Pradesh, Karnataka, Tamil Nadu, and Kerala; (ii) Western Region: Gujarat and Maharashtra; (iii) Northern Region: Haryana, Himachal Pradesh, Jammu & Kashmir, Punjab, and Rajasthan; (iv) North-Eastern Region: Assam; (v) Central Region: Uttar Pradesh and Madhya Pradesh; (vi) Eastern Region: Bihar, Odisha, and West Bengal.

Fertilizer consumption, gross irrigated area, gross sown area, net irrigated area, net sown area, food-grain yield, oilseed yield, food-grain area, oilseed area, forest area, permanent pasture, and grazing lands, land not available for cultivation and cropping intensity were derived from the Centre for Monitoring Indian Economy (CMIE), Ministry of Agriculture and Farmers Welfare, Government of India (GoI). The average size of land holdings was taken from the Agriculture Census, Department of Agriculture, Co-operation & Farmers Welfare (GoI). Per capita availability of milk production was taken from the Department of Animal Husbandry, Dairying & Fisheries, Ministry of Agriculture, GoI. The credit Deposit Ratio (CDR) of scheduled commercial banks, credit disbursed to agriculture by scheduled commercial banks, and agriculture Gross Domestic Product (GDP) was taken from the Reserve Bank of India, GoI. Gender ratio, population density, population growth, and urbanization were taken from Census, GoI. Rural literate population, rural poor population, Gini coefficient of distribution of consumption, total literacy rate, and female literacy rate were taken from Niti Ayog, GoI. Birth and infant mortality rates were taken from the Office of the Registrar General and Census, GoI. Per capita net state domestic product, per capita availability of food-grain production, road length, and government expenditure on the social sector were derived from the website of RBI, Central Statistics Office, GoI. Annual average precipitation, Annual Average Maximum and Minimum Temperature (AAMaxT and AAMinT), and Actual Annual Rainfall (AARF) were taken from GIS online database and Indian Metrological Department (IMD), GoI. Data for a few variables (e.g., literacy rate, female literacy rate, birth rate, urbanization, population density, the average size of land holding, rural literate person, rural poor people) were not available in the time series. Thus, interpolation and extrapolation methods were used to compute the median values of these variables to complete the time series of 1990-2017 (Kumar et al. 2017; Singh et al. 2019).

Theoretical foundation on measurement of agricultural sustainability

Previous studies have claimed that an index-based estimation is an effective tool for assessing agricultural sustainability (Zhen and Routray 2003; Sharma and Shardendu 2011; Fallah-Alipour et al. 2018; Talukder et al. 2020). The approach is useful for formulating agricultural

development policies and comparing agricultural sustainability across regions (Valizadeh and Hayati, 2021). However, different indicators have been used to create ASI in various economies using micro and macro-level information. Therefore, there is no consistent process for measuring agricultural sustainability and defining its major indicators in the existing literature (Roy and Chan 2012; Lampridi et al. 2019). Aggregation of several factors as an index for agricultural sustainability assessment was introduced by the World Bank, United Nations, and Organization for Economic Cooperation and Development (OECD) in the 1970s (Gaetano 2010). However, existing studies have developed several indexes such as integrated sustainability score, farm assessment index, ASI, farmers development index, sustainable livelihood security index, and agricultural sustainability measurement index to assess the performance of agricultural sustainability using primary and secondary data (Qiu et al. 2007; Hatai and Sen 2008; Gaetano 2010; Sharma and Shardendu 2011; Roy and Chan 2012; Rostami and Mohammadi 2017; Kareemulla et al. 2017; Fallah-Alipour et al. 2018; Mili and Martínez-Vega 2019; Talukder et al. 2020; Valizadeh and Hayati 2021). Most studies have used simple descriptive, principal components, and factor component analysis, including the normalization values of a selected set of variables. In this, a study following processes was used to develop ASI:

Segregation of indicators

Agricultural sustainability integrates economic efficiency, social equity, and ecological security-related variables (Gaetano 2010; Fallah-Alipour et al. 2018). Thus, selected indicators were divided into the categories mentioned earlier.

Estimation of Composite Z-Score

It converts all values of a specific variable between 0-1 and makes relative comparisons across entities (Gaetano 2010; Fallah-Alipour et al. 2018). For example, if a variable had a positive impact on agricultural sustainability as per the available theoretical literature, then Composite Z-Score (CZS) (Kareemulla et al. 2017; Rostami and Mohammadi 2017) was estimated as follows:

$$CZS_{is} = \{[X_{is} – Min(X_{is})]/[Max(X_{is}) – Min(X_{is})]\} \quad (1)$$

Here, CZS is the Composite Z-Score for the i^{th} variable, and s is cross-sectional states. X_{is} is the actual value; $Min(X_{is})$ is the minimum value; $Max(X_{is})$ is the highest value for a specific variable across states in equation (1). Values of CZS for a specific variable lie between 0-1. If a factor had a negative impact on agricultural sustainability according to existing literature, then the CZS (Rostami and Mohammadi 2017) was estimated as follows:

$$CZS_{is} = \{[X_{is} – Max(X_{is})]/[Min(X_{is}) – Max(X_{is})]\} \quad (2)$$

Clarification of all variables is given in equation (1).

Estimation of weights for arbitrary variable

The Weightage technique is useful for dividing the indicators into positive or endogenous and negative or exogenous (Fallah-Alipour et al. 2018). In this study, weightage for each factor (Kumar et al. 2017; Singh and Issac 2018; Singh et al. 2019) was assigned as follows:

$$Wi = \frac{K}{\sqrt{Var(CZS)}} \quad (3)$$

Here, W_i is weightage ($0<W>1$ assigned to i^{th} variable and $\sum_{i=1}^{n} Wi = 1$). $Var(CZS)$ is a statistical variation across Composite Z-Scores for all variables in equation (3). K was measured as follows:

$$\text{Here,} K = \frac{1}{\left\{\sum_{i=1}^{n}\left(\frac{1}{\sqrt{Var(CZS)}}\right)\right\}} \quad (4)$$

Aggregate sum

It is a linear average sum of all CZS multiplied by assigned weights under a specific measurement category.

Development of the Agricultural Sustainability Index (ASI)

Agricultural sustainability has a multidimensional and complex association with all activities in a country (Valizadeh and Hayati, 2021). So, agricultural sustainability assessment is controversial (Hatai and Sen 2008; Sydorovych and Wossink 2008; Fallah-Alipour et al. 2018; Lampridi et al. 2019; Mili and Martínez-Vega 2019; Talukder et al. 2020). Existing researchers do not have unanimity on agricultural sustainability (Kareemulla et al. 2017). Current studies have also observed that agricultural sustainability is an integrated component of social, economic, and ecological sustainability (Sharma and Shardendu 2011; Latruffe et al. 2016; Ryan et al. 2016; Lampridi et al. 2019). Accordingly, EEI, SEI, and ESI can be developed to examine agricultural sustainability. Subsequently, in this study, ASI was considered as a linear average sum of EEI, SEI, and ESI, which was estimated as follows:

$$(ASI)_{st} = \{(EEI)_{st} + SEI)_{st} + (ESI)_{st}\}/3 \quad (5)$$

Here, ASI is Agricultural Sustainability Index, EEI is Economic Efficiency Index, SEI is the social equality index, and ESI is Ecological Security Index in equation (5).

Economic Efficiency Index (EEI)

A single variable may not explain economic efficiency. Thus, this study used per capita GDP as the most useful and effective representative variable for economic development. However, economic efficiency or development is a multidimensional concept and has a significant association with several country activities (Latruffe et al. 2016). Few studies have developed Economic Development Index (EDI) to assess the relative performance of economic development across countries. It is helpful for farmers to increase their profitability in the agricultural sector (Gaetano 2010). Thus, economic

development helps increase the agricultural production system's productivity, profitability, and stability (Zhen and Routray, 2003; Latruffe et al. 2016). Therefore, this study has formulated EEI to investigate the relative economic efficiency of selected Indian states. Here, EEI was considered as a function of per capita net state domestic product, CDR of scheduled commercial banks, the ratio of credit to agriculture by scheduled commercial banks with the gross sown area, a ratio of agriculture GDP with the gross sown area, a ratio of a gross irrigated area with the gross sown area, a ratio of a net irrigated area with the net sown area, the average size of land holdings, a yield of food-grain and oilseeds crops, percentage area under food-grain and oilseeds crops, a ratio of the rural literate population with gross sown area and ratio of the rural poor population with the gross sown area. EEI was estimated as a linear sum of CZS of all associated variables that were multiplied by assigned weightages and explained as:

$$(EEI)_{st} = W_1\times(CZS\_PCNSDP)_{st} + W_2\times(CZS\_CDR)_{st} + W_3\times(CZS\_CASCB/GSA)_{st} + W_4\times (CZS\_AGDPGSA)_{st} + W_5\times(CZS\_GIA/GSA)_{st} + W_6\times(CZS\_NIA/NSA)_{st} + W_7\times(CZS\_ASLH)_{st} + W_8 \times(CZS\_TFGY)_{st} + W_9\times(CZS\_TOSY)_{st} + W_{10}\times(CZS\_FGAPGSA)_{st} + W_{11}\times(CZS\_OASPGSA)_{st} + W_{12}\times(CZS\_RLP/GSA)_{st} + W_{13}\times(CZS\_RPPGSA)_{st} \quad (6)$$

Here, W_1 …. W_{13} are the assigned weightages, and CZS is the Composite Z-Score of associated variables in equation (6). A brief of economic efficiency-associated variables has been given in Table 1.

Per capita income is a vital indicator of national development and prosperity (Hatai and Sen, 2008). It also maintains overall livelihood security and agricultural sustainability. Thus, per capita net state domestic product was considered to estimate EEI (Singh and Issac 2018). The CDR helps increase money flow and financial stability in the domestic market and is a vibrant determinant of economic efficiency. Credit disbursement to the agricultural sector contributes to increasing agricultural production (Kumar et al. 2017). Value of show per hectare land is also helpful in increasing economic efficiency (Hensen 1996; Gaetano 2010; Latruffe et al. 2016; Singh and Issac 2018; Mili and Martínez-Vega 2019). Thus, a ratio of agriculture GDP with the gross sown area was used to estimate EEI. Irrigated area has high yielding capacity in cultivation (Kumar et al. 2017; Singh and Issac 2018). Hence, a ratio of the gross irrigated area with the gross sown area and the net irrigated area with the net sown area was used to develop EEI (Ghabru et al. 2017). Farm management practices and technologies can be used in large landholding. Thus, landholding size is a vital contribution to increasing agricultural sustainability (Hensen 1996; Gaetano 2010; Fallah-Alipour et al. 2018; Mili and Martínez-Vega 2019). High yields of food-grain and oilseed crops are the fruit of better soil fertility and quality, irrigation, and technological advancement (Hatai and Sen 2008; Kareemulla et al. 2017; Singh and Issac 2018). It also increases the farmers' profitability; thus, it is a crucial determinant of agricultural sustainability (Ghabru et al. 2017). The cropped area under food-grain and oilseed crops greatly contributes to agricultural sustainability (Kumar et al. 2017; Mili and Martínez-Vega 2019). Though India is rich in traditional knowledge of agriculture, a literate person understands modern agricultural technologies, irrigation methods, appropriate time of planting and irrigation, and adaptation strategies to climate change in farming (Kumar et al. 2016). Thus, agricultural sustainability increases with an increase in the participation of the literate population in cultivation (Kumar et al. 2017; Talukder et al. 2020). On the contrary, poor farmers cannot use various practices in cultivation due to their financial restrictions (Kumar et al. 2017). Thus, the role of poor farmers may be harmful to agricultural sustainability.

Table 1. Explanation of economic efficiency associated variables

Indicator	Unit	Symbol	Expected sign
Per capita net state domestic product at factor cost (at current prices)	Rs	*PCNSDP*	Positive
Credit Deposit Ratio (GDP) of scheduled commercial banks according to a place of utilization	%	*CDR*	Positive
Ratio of credit to agriculture by scheduled commercial banks with gross sown area	Rs/Ha	*CASCB/GSA*	Positive
Ratio of agriculture GDP with gross sown area	Rs/Ha	*AGDPGSA*	Positive
Ratio of gross irrigated area with gross sown area	Ratio	*GIA/GSA*	Positive
Ratio of net irrigated area with net sown area	Ratio	*NIA/NSA*	Positive
Average size of holdings	Ha/Holding	*ASLH*	Positive
Total food-grain yield	Kg/Ha	*TFGY*	Positive
Total oilseeds yield (nine crops)	Kg/Ha	*TOSY*	Positive
Food-grain area as % of gross sown area	%	*FGAPGSA*	Positive
Oilseeds area as % of gross sown area	%	*OASPGSA*	Positive
Ratio of rural literate population with gross sown area	Number	*RLP/GSA*	Positive
Ratio of rural poor population with gross sown area	Number	*RPPGSA*	Negative

Social Equity Index (SEI)

Social development is a multidimensional concept that may not be defined by a specific variable (Singh et al. 2019). Human capital and communication among men and women increase as social equity increases (Gaetano 2010). Thus, social equity improves as an increase in factors related to social development (Zhen and Routray 2003). Previous studies developed SEI to examine the ASI in different countries (Hatai and Sen 2008; Gaetano 2010; Sharma and Shardendu 2011; Ghabru et al. 2017; Kareemulla et al. 2017; Fallah-Alipour et al. 2018). In this study, SEI was considered as a function of per capita availability of food-grain and milk production, literacy rate, female literacy rate, gender ratio, birth rate, infant mortality rate, road length per 1000-person, Gini coefficient of distribution of consumption (rural area) and per capita expenditure on the social sector. The SEI was estimated as a linear sum of CZS of all related variables multiplied by an assigned weight and described as:

$$(SEI)_{st} = W_1\times(CZS\_PCAFGP)_{st} + W_2\times(CZS\_PCAMP)_{st} + W_3\times(CZS\_TLR)_{st} + W_4\times(CZS\_FLRRU)_{st} + W_5\times(CZS\_GenRat)_{st} + W_6\times(CZS\_BRRU)_{st} + W_7\times(CZS\_IMR)_{st} + W_8\times(CZS\_RLPTP)_{st} + W_9\times(CZS\_GCDCRA)_{st} + W_{10}\times(CZS\_PCESS) \quad (7)$$

Here, W_1 …. W_{10} are the allocated weightages, CZS is the Composite Z-Score of associated variables, and SEI is the Social Equity Index in equation (7). A brief explanation of the variables is given in Table 2.

Per capita availability of food-grain and milk production significantly contributes to increasing social equity (Zhen and Routray 2003; Singh and Hiremath 2010; Ghabru et al. 2017; Singh and Issac 2018). These variables help increase food security, human health, and social equity. Moreover, education level is a vibrant determinant of increasing social equity and agricultural sustainability (Latruffe et al. 2016; Kareemulla et al. 2017; Fallah-Alipour et al. 2018). Furthermore, female literacy measures the overall performance of women's empowerment (Hatai and Sen 2008; Ghabru et al. 2017). It is also helpful for population stabilization and maintaining social equity (Singh and Issac 2018). Gender equality indicates social equity and women's development (Gaetano 2010; Latruffe et al. 2016). For example, the birth rate significantly impacts economic development, urbanization, social structure, and religion. Thus, it may be a useful determinant of social equity (Singh and Issac 2018). Infant mortality rate infers women's overall performance and health security and the impact of medical facilities on social security (Hensen 1996; Hatai and Sen 2008; Latruffe et al. 2016; Ghabru et al. 2017; Singh and Issac 2018). Road connectivity measures the progress of infrastructural development, making transportation easy for people. Thus, it significantly contributes to social development (Hatai and Sen 2008; Kumar et al. 2017). Equal income distribution effectively maintains social equality (Zhen and Routray 2003; Kumar et al. 2017). Public expenditure on the social sector is also helpful in increasing social equity (Talukder et al. 2020). Therefore, the abovementioned variables were used to develop SEI in this study.

Ecological Security Index (ESI)

Ecological security helps develop a natural resource-based economy (Ghabru et al. 2017). It maintains the land use pattern, biodiversity, forest area, groundwater, soil fertility and quality, and air quality (Fallah-Alipour et al. 2018). Accordingly, it contributes to increasing agricultural sustainability. Water availability and soil fertility are important determinants of agricultural sustainability (Zhen and Routray, 2003). Biodiversity conservation and environmental protection are essential to increase agricultural sustainability (Mili and Martínez-Vega 2019). Hence, ecological security may not be evaluated by a single activity. Singh et al. (2019) created Environmental Sustainability Index (EnSI) to assess the environmental performance across countries. Rostami and Mohammadi (2017) and Mili and Martínez-Vega (2019) generated ESI to assess the performance of agricultural sustainability. Hence, in this study, ESI was formulated as a composition of the ratio of forest area with the gross sown area, a ratio of permanent pasture and grazing lands with the net sown area, the ratio of land not available for cultivation with the gross sown area, cropping intensity, fertilizer consumption/hectare land, population density, population growth, percentage population living in an urban area and annual average precipitation. The ESI was estimated as a linear sum of CZS of all associated variables multiplied by an assigned weight and explained as:

$$(ESI)_{st} = W_1\times(CZS\_RFAGSA)_{st} + W_2\times(CZS\_RPPGLNSA)_{st} + W_3\times(CZS\_RLNACGSA)_{st} + W_4\times(CZS\_CroInt)_{st} + W_5\times(CZS\_FCPHL)_{st} + W_6\times(CZS\_PopDen)_{st} + W_7\times(CZS\_PGR)_{st} + W_8\times(CZS\_UR)_{st} + W_9\times(CZS\_AAPCP)_{st} \quad (8)$$

Here, ESI is Ecological Security Index; W_1 …. W_9 are the allocated weightages of corresponding variables; and CZS is Composite Z-Score of associated variables in equation (8). The explanation of other variables is given in Table 3.

Forest areas, permanent pastures, and grazing land are essential to sustain environmental quality (Ghabru et al. 2017). Also, forest area absorbs CO_2 emissions from various production sources, and it is helpful to maintain air quality and ecological services. Thus, these variables have a positive impact on agricultural sustainability. Therefore, the ratio of forest area with the gross sown area and the ratio of permanent pasture and grazing lands with the net sown area were considered to estimate the ESI (Singh and Issac 2018; Singh et al. 2019). Not cultivated land for farming has a negative implication on agricultural sustainability. Thus, the ratio of land unavailable for cultivation with the gross sown area was used to develop ESI (Mili and Martínez-Vega 2019). Cropping intensity measures a particular land's use for growing various crops in a year. The production of food grain and commercial crops and farmers' income increase as cropping intensity increases. Consequently, it positively impacts agricultural sustainability (Kumar et al. 2017; Singh and Issac 2018). The application of fertilizer and pesticides in cultivation may be caused to increase in environmental degradation (Lampridi et al. 2019). Thus, agricultural sustainability may be adversely affected due to the extensive use of fertilizer in the agricultural sector (Singh et al. 2019).

Table 2. Explanation of social equity associated variables

Indicator	Unit	Symbol	Expected sign
Per capita availability of food-grain production	Kg./Year	*PCAFGP*	Positive
Per capita availability of milk production	Gram/day	*PCAMP*	Positive
Total literacy rate (Rural+Urban)	%	*TLR*	Positive
Female literacy rate (Rural+Urban)	%	*FLRRU*	Positive
Gender ratio (Female/1000 Males)	Number	*GenRat*	Positive
Birth rate (Rural+Urban) (Per '000 population)	Number	*BRRU*	Positive
Infant mortality rate (Per '000 live births)	Number	*IMR*	Negative
Road length per 1000 person	Km/1000 person	*RLPTP*	Positive
Gini coefficient of distribution of consumption (Rural+Urban)	Number	*GCDCRA*	Negative
Per capita expenditure on social sector (Rural+Urban)	Rs	*PCESS*	Positive

Table 3. Explanation of ecological security associated variables

Indicators	Unit	Symbol	Expected sign
Ratio of forest area with gross sown area	Ratio	*RFAGSA*	Positive
Ratio of permanent pasture and grazing lands with net sown area	%	*RPPGLNSA*	Positive
Ratio of land not available for cultivation with gross sown area	%	*RLNACGSA*	Negative
Cropping intensity	%	*CroInt*	Positive
Fertilizer consumption/hectare land (N+P+K)	Kg	*FCPHL*	Negative
Population density	Number	*PopDen*	Negative
Population growth rate (Rural+Urban)	%	*PGR*	Negative
Percentage population living in an urban area (Urbanization)	%	*UR*	Negative
Annual average precipitation	mm	*AAPCP*	Positive

Moreover, ecosystem services are negatively impacted due to overwhelming population density, population growth, and urbanization. Therefore, these factors increase the additional pressure on ecological services (Fallah-Alipour et al. 2018; Singh et al. 2019). On the other hand, precipitation is a natural resource that contributes to increasing and sustaining agricultural production. Hence, as mentioned earlier, this study used those variables to estimate ESI.

Empirical model on the association of ASI with climatic factors

Previous studies could not examine the impact of climatic factors on agricultural sustainability. Hence, this study examines the influence of climatic factors (i.e., AAMaxT, AAMinT, and AARF) on ASI. For the investigation above, the present study adopted a model from studies of Kumar et al. (2017), Singh and Issac (2018), and Singh et al. (2019), which used estimated indexes as dependent and independent variables. Therefore, a linear regression model was used to estimate the regression coefficient of explanatory variables with ASI and specified as:

$$(ASI)_{st} = \alpha_0 + \alpha_1 (TTF)_{st} + \alpha_2 (EEI)_{st} + \alpha_3 (SEI)_{st} + \alpha_4 (ESI)_{st} + \alpha_5 (AAMaxT)_{st} + \alpha_6 (AAMinT)_{st} + \alpha_6 (AARF)_{st} + \lambda_{st} \quad (9)$$

Here, ASI is the Agricultural Sustainability Index, EEI is Economic Efficiency Index, SEI is Social Equity Index; AAMaxT and AAMinT are the Annual Average Maximum And Minimum Temperature, respectively; AARF is the Actual Annual Rainfall; TTF is the time trend factor that was used to capture the influence of technological advancement on agricultural sustainability (Kumar et al. 2017); α_0 is the constant coefficient; $\alpha_1, \ldots, \alpha_6$ are the regression coefficients of associated independent variables; λ_{st} is the error term; and *s* is cross-sectional states; *t* is period in equation (9). Log-linear and non-linear regression models were also used to check the consistency of regression coefficients.

RESULTS AND DISCUSSION

Presentation of Indian States in economic efficiency

The mean values of EEI during 1990-2017 are given in Figure 1. It revealed that Haryana and Punjab were 1st and 2nd in the position, respectively, regarding economic efficiency among the 17 Indian states. These states have a better position in irrigated areas, yield of food-grain and oilseed crops, literate population, cropping intensity, per capita net state domestic product, and credit facilities for the agricultural sector used to develop EEI. Therefore, Punjab and Haryana were in the best position for agricultural sustainability. EEI values for Tamil Nadu, Gujarat, West Bengal, and Andhra Pradesh were between 0.30 to 0.40. Thus, these states have the 3rd, 4th, 5th, and 6th positions concerning economic efficiency. The EEI values for Kerala, Rajasthan, Karnataka, Uttar Pradesh, Madhya Pradesh, Maharashtra, Jammu & Kashmir were between 0.20 to 0.30. Thus, these states have a relatively poor position regarding economic efficiency. Regarding economic efficiency, Himachal Pradesh, Bihar, and Assam have the 15th, 16th, and 17th positions. Furthermore, the value of EEI lies between 0.17 to 0.82 across Indian states. Thus, the estimates showed a significant variation in

economic efficiency across Indian states due to the high diversity in economic development-related variables.

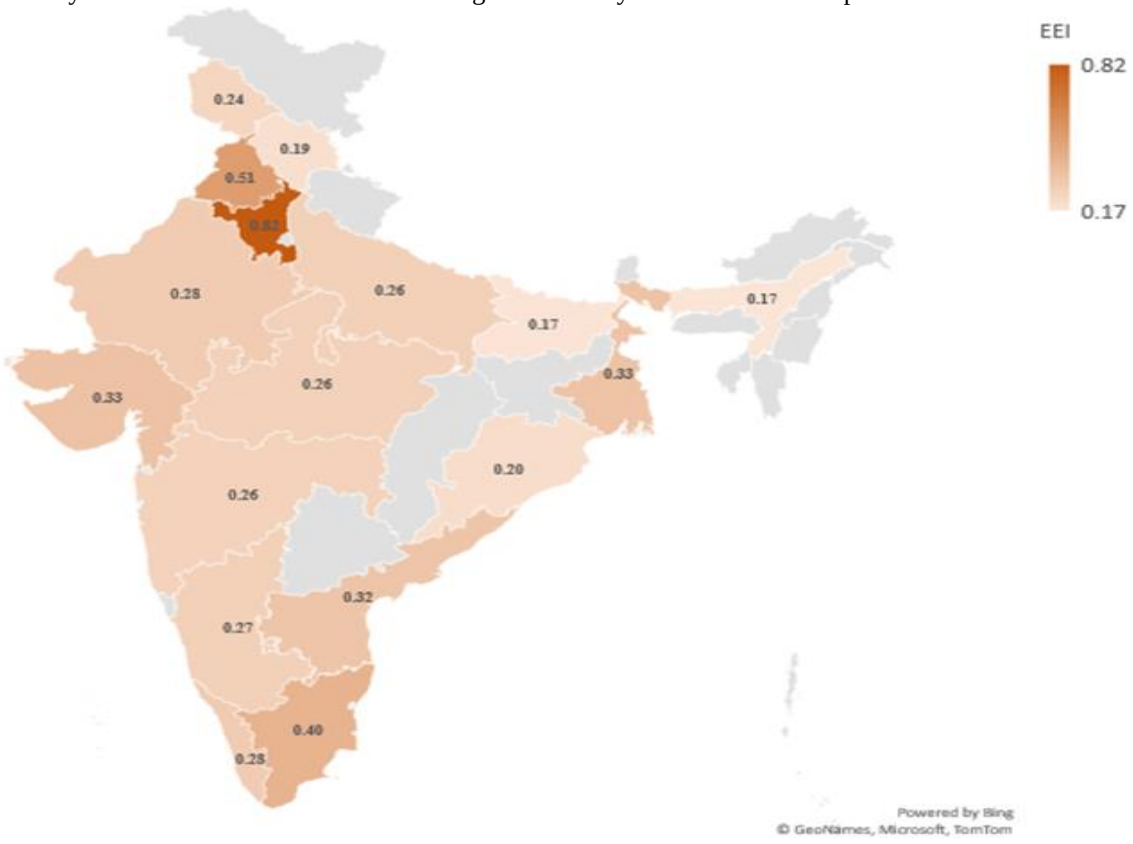

Figure 1. Comparison of Indian states as per the Economic Efficiency Index (EEI)

Performance of Indian states in social equity

A comparison of Indian states based on estimated mean values of an SEI during 1990-2017 is given in Figure 2. Kerala and Himachal Pradesh have shown 1st and 2nd positions in social equity. Moreover, the values of SEI were an integrated index of various variables which have a significant association with social development. Kerala and Himachal Pradesh have high literacy rates, female literacy rates, gender ratios, and per capita expenditure in the social sector. Thus, both states have a better position in social equity among the Indian states. On the other hand, per capita availability of food-grain and milk production was higher in Punjab and Haryana than in other Indian states. Thus, Punjab and Haryana have the 3rd and 4th positions in social equity among the 17 Indian states. Maharashtra, Tamil Nadu, Gujarat, Assam, Rajasthan, Odisha, West Bengal, Jammu & Kashmir, Madhya Pradesh, and Andhra Pradesh have a relatively poor position in social equity. Bihar and Uttar Pradesh seemed to worsen their position in social equity among the Indian states. Bihar and Uttar Pradesh have low per capita availability of food-grain production, per expenditure on social sector and milk production, high infant mortality rate, and inequality in consumption pattern. Thus, these states could not improve their position in social equity. Furthermore, high variation in social equity was experienced due to significant diversity in social development-related activities in Indian states.

Performance of Indian states in ecological security

The cross comparative of Indian states in ecological security based on mean values of ESI during 1990-2017 is given in Figure 3. It infers that Himachal Pradesh was in the best position in ecological security among the 17 Indian states. The values of ESI lie between 0.72-0.26 across Indian states. It indicates a high variation in ecological security across the Indian states. As the value of ESI was an integrated index of share of forest area, permanent pasture and grazing land not available for cultivation in the gross sown area; cropping intensity; fertilizer consumption; population density; population growth; urbanization; and annual average precipitation. Himachal Pradesh has shown a better position in most factors positively associated with ecological security. That means the state has maintained its significant position in ecological security. On the other hand, Odisha ranked 2nd in ecological security due to its better forest area, permanent pasture and grazing lands, and annual precipitation. Gujarat, Tamil Nadu, and Haryana have ranked 15th, 16th, and 17th in ecological security. Thus, these states could not improve their position in ecological security. The ESI values lie between 0.4-0.5 for Assam, Jammu & Kashmir, Kerala, Madhya Pradesh, and West Bengal. These states were in a moderate position in ecological security among the Indian states.

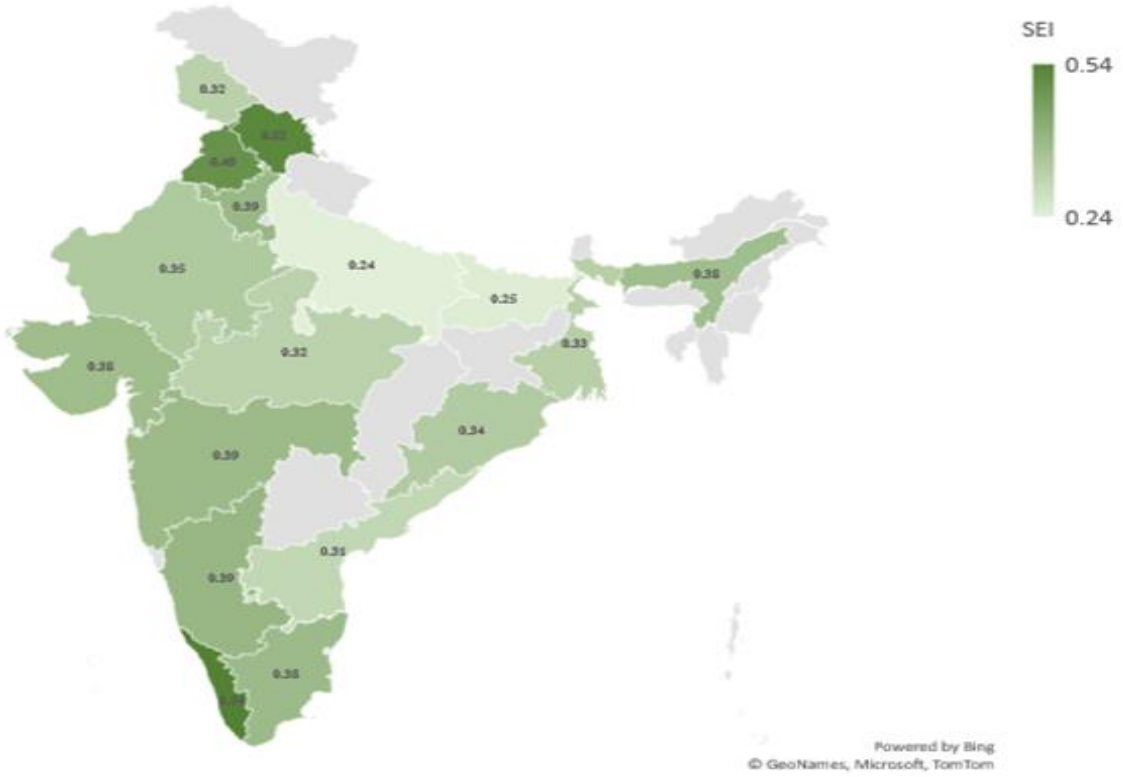

Figure 2. Comparison of Indian states based on the Social Equity Index (SEI)

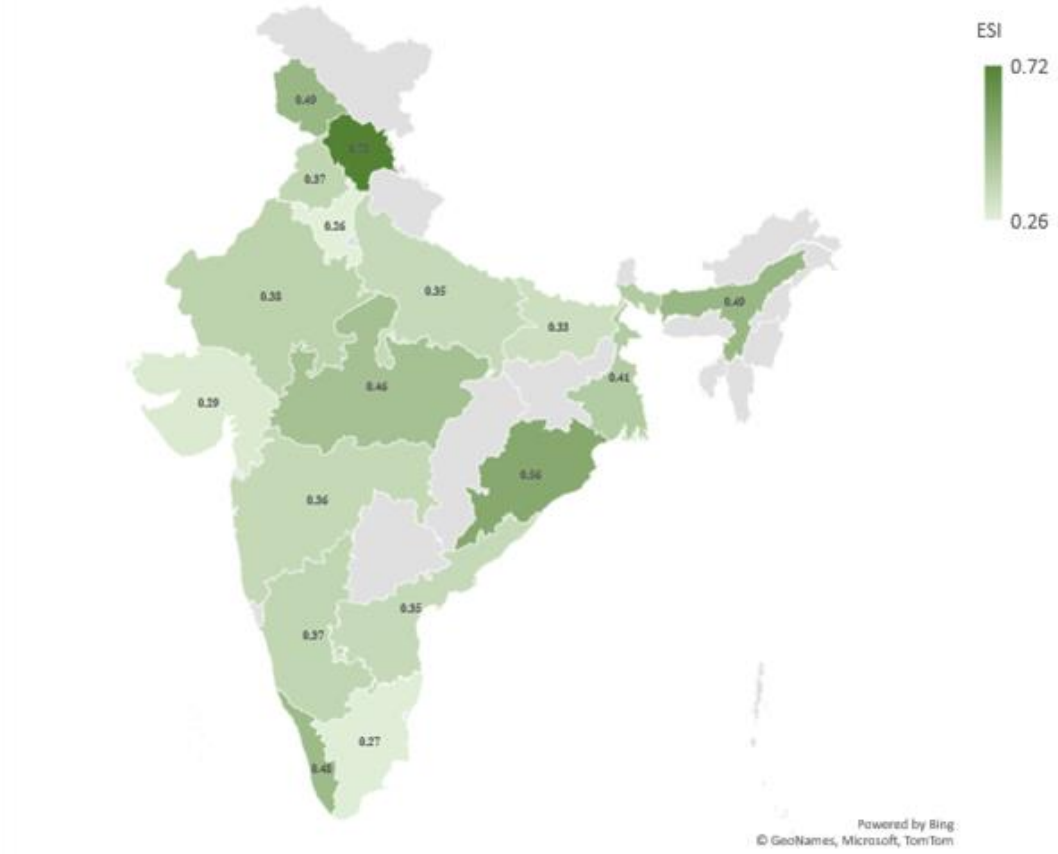

Figure 3: Comparison of Indian states based on the Ecological Security Index (ESI)

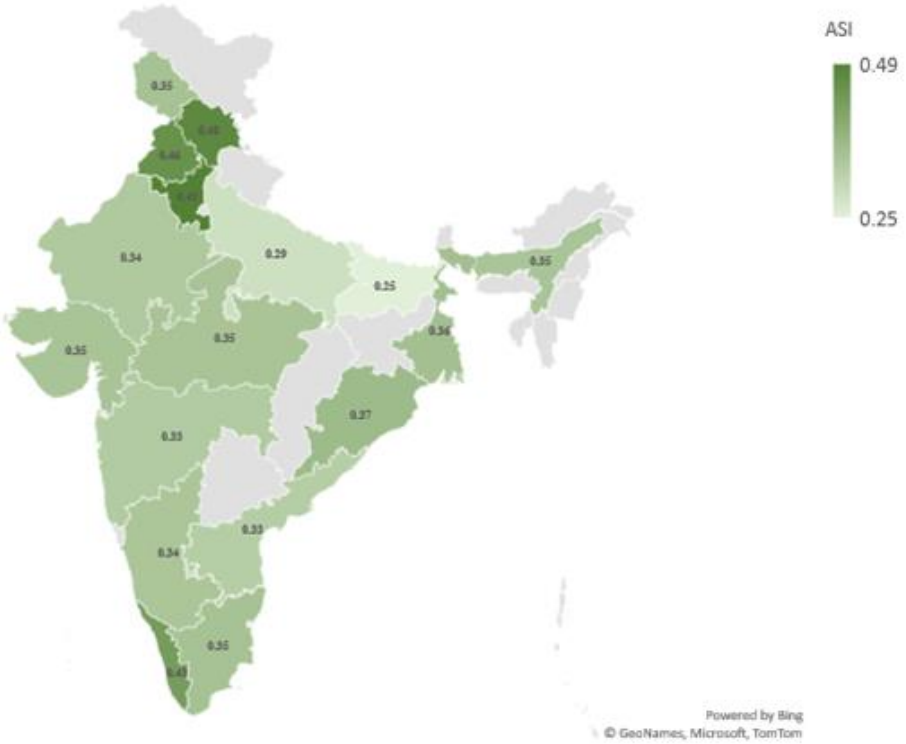

Figure 4. Performance of Indian states in agricultural sustainability

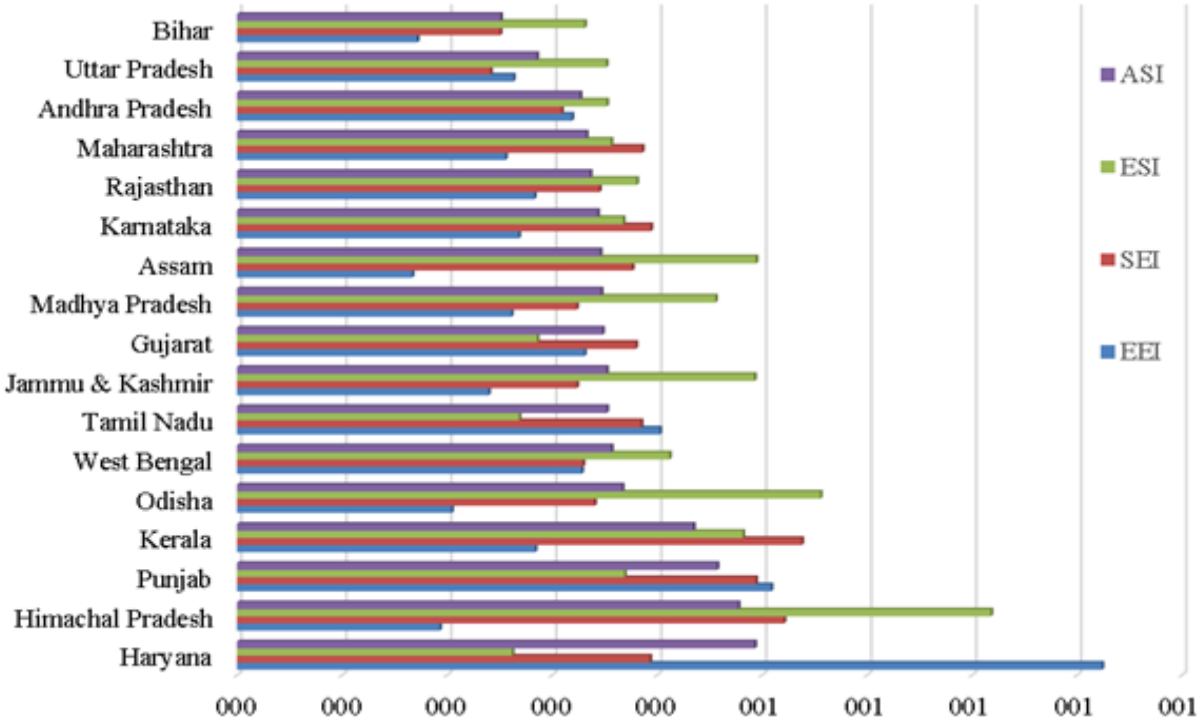

Figure 5. Comparison between states based on EEI, SEI, ESI, and ASI

Performance of Indian States in agricultural sustainability

The cross-comparison of Indian states in agricultural sustainability as per the estimated mean values of ASI from 1990 to 2017 is given in Figure 4. Cross comparison of states based on EEI, SEI, ESI, and ASI is given in Figure 5. The values of ASI lie between 0.25-0.49. It infers that there was high variation in agricultural sustainability across Indian states. Haryana, Himachal Pradesh, and Punjab have ranked 1st, 2nd, and 3rd in ASI, respectively. These two states have the better position in most factors which were the main determinants of economic efficiency, social equity, and ecological security. It means that these states have the appropriate ecosystem to maintain agricultural sustainability. On the other hand, Bihar and Uttar Pradesh have the 17th and 18th ranks in ASI. Thus, both states could not maintain agricultural sustainability due to several reasons such as extreme poverty, low literacy rate, high pressure of population on agriculture, low cropping intensity, high-income inequality, high unemployment rate, low infrastructural development, and others.

Validity of ASI, EEI, SEI, and ESI

Internal and external validation of an index is essential to increase the unanimity among the researchers and academicians. Thereupon, an estimated index can be used for further empirical investigation. Therefore, the Karl-Pearson correlation coefficient of ASI with EEI, SEI, and ESI was estimated to check their internal validity. The correlation coefficients of these indexes with climatic factors were assessed to check their external validity. Kumar et al. (2017), Singh and Issac (2018), and Singh et al. (2019) have also used a similar technique to identify the internal and external validity of purposed indexes. The correlation coefficient of ASI with EEI, SEI, ESI, AAMaxt, and AAMinT were statistically significant at a 1% significant level (Table 4). Thus, these indexes' viability and estimates infer that agricultural sustainability increases with economic efficiency, social equity, and ecological security. On the other hand, annual maximum and minimum temperatures were negatively associated with agricultural sustainability. Here, climate change seemed to harm agricultural sustainability in India. It was also reported that agricultural sustainability could not be achieved without maintaining economic efficiency, social equity, and ecological security in the agricultural sector.

Discussion on empirical results

The empirical results which examine the influence of EEI, SEI, ESI, AAMaxt, AAMinT, and AARF on ASI are given in Table 5. The regression coefficient of the variables above with ASI was estimated through linear, log-linear, and non-linear regression models. Furthermore, the panel correction standard estimation model effectively reduced the incidence of serial correlation, heteroskedasticity, and cross-sectional autocorrelation in panel data investigation (Kumar et al. 2016, 2017; Singh et al. 2019). Thus, this model was considered to estimate the regression coefficient of the aforementioned independent variables with ASI. The mean values of the Variance Inflation Factor (VIF) for linear and log-linear regression models were 3.21 and 5.36, respectively, indicating the absence of multi-correlation among the explanatory variables in panel data. Furthermore, χ^2 values under the Ramsey RESET test for powers of the fitted values of ASI and the independent variables were statistically significant at a 1% significance level. Hence, the functional form of the proposed models was found to be well-defined. A log-linear regression model is reported to have a lower value of Akaike Information Criterion (AIC) and Bayesian Information Criteria (BIC) as compared to linear and non-linear regression models (Kumar et al. 2017; Singh and Issac 2018; Singh et al. 2019). Hence, the explanation of results based on this model was included in this study.

The R2 value was 0.94, showing that 94% of the variation in agricultural sustainability depends on technological advancement, economic efficiency, social equity, ecological security, and climatic factors. The regression coefficient of the time trend factor with agricultural sustainability appeared positive and statistically significant, indicating that technological advancement in farming would be useful to increase crop production and agricultural sustainability. The regression coefficient of EEI, SEI, and ESI with ASI seemed positive and statistically significant. The estimates showed that agricultural sustainability improved with increased economic efficiency, social equity, and ecological security. On the other hand, the AAMaxT negatively influenced ASI. Thus, it was seen that agricultural sustainability might decline with an increase in AAMaxT. While agricultural sustainability was positively associated with AAMinT and AARF in India.

Table 4. The correlation coefficient of ASI with its components and climatic factors

	ASI	**EEI**	**SEI**	**ESI**	**AAMaxT**	**AAMinT**	**AARF**
ASI	1						
EEI	0.563**	1					
SEI	0.799**	0.199**	1				
ESI	0.334**	-0.510**	0.348**	1			
AAMaxT	-0.358**	0.145**	-0.293**	-0.601**	1		
AAMinT	-0.338**	0.017	-0.182**	-0.482**	0.895**	1	
AARF	0.016	-0.212**	0.112**	0.230**	-0.042	0.122**	1

Note: **: Correlation coefficient is statistically significant at the 0.01 level. ASI: Agricultural Sustainability Index; EEI: Economic Efficiency Index; SEI: Social Equity Index; ESI: Ecological Security Index; AAMaxT: Annual Average Maximum Temperature; AAMinT: Annual Average Minimum Temperature; AARF: Annual Actual Rainfall

Table 5. Association of ASI with its components and climatic factors

Name of models	Linear regression		Log-linear regression		Non-linear regression	
No. of Obs.	476		476		476	
VIF	3.21		5.36		489.66	
AIC	-3048.007		-1626.31		-3044.36	
BIC	-3014.684		-1592.99		-2994.38	
Ramsey RESET test using powers of the fitted values of *ASI*	0.69		2.98		0.05	
Ramsey RESET test using powers of the independent variables	1.4		43.56		1.32	
R^2	0.9786		0.9419		0.979	
Wald $\chi^2(7)$	1.01E+06		3.49E+04		1.79E+06	
Prob > χ^2	0.000		0.000		0.000	
ASI	Reg. Coef.	P>|z|	Reg. Coef.	P>|z|	Reg. Coef.	P>|z|
TTF	0.0001	0.001	0.0005	0.002	0.0001	0.009
EEI	0.3284	0.000	0.3389	0.000	0.3342	0.000
(EEI)^2	-	-	-	-	-0.0044	0.677
SEI	0.3430	0.000	0.3581	0.000	0.3597	0.000
(SEI)^2	-	-	-	-	-0.0230	0.532
ESI	0.3217	0.000	0.3548	0.000	0.3029	0.000
(ESI)^2	-	-	-	-	0.0221	0.466
AAMaxT	-0.0001	0.811	-0.1414	0.002	-0.0027	0.732
(AAMaxT)2	-	-	-	-	0.0001	0.694
AAMinT	0.0001	0.570	0.0060	0.799	-0.0029	0.321
(AAMinT)^2	-	-	-	-	0.0001	0.287
AARF	0.0000	0.007	0.0060	0.029	0.0001	0.803
(AARF)^2	-	-	-	-	-0.0001	0.836
Con. Coef.	-0.2870	0.001	-0.4337	0.201	-0.2144	0.178

Note: VIF: Variance Inflation Factor; AIC: Akaike Information Criterion; BIC: Bayesian Information Criteria; Con. Coef.: Constant Coefficient

The regression results based on a non-linear regression model showed that agricultural sustainability was non-linearly associated with economic efficiency, social equity, AAMaxT and AAMinT, and AARF. Economic efficiency, social equity, and AARF had a U-shaped function with agricultural sustainability. Thus, it also indicated a decrease in agricultural sustainability with an increase in economic efficiency, social equity, and rainfall to a certain extent. Agricultural sustainability showed a hilly-shaped relationship with AAMaxT and AAMinT. The estimates provided evidence that annual maximum and minimum temperatures positively affect agricultural sustainability up to a certain level; thereafter, both variables will harm it. Finally, ecological security had a linear relationship with agricultural sustainability, indicating improved agricultural sustainability with increased ecological security.

Conclusion and policy suggestions

The descriptive results showed that ASI was positively associated with EEI, SEI, and ESI. Haryana and Himachal Pradesh had the 1st and 2nd ranks in ASI. On the other hand, Bihar, Uttar Pradesh, and Andhra Pradesh had the 17th, 16th, and 15th ranks, respectively, in ASI. Hence, the agriculture sector was in a vulnerable position in these states. Thus, economic development, social development, and ecological security factors may be useful to increase India's agricultural sustainability. Therefore, there is necessary to maintain sustainability in economic, social, and environmental development to improve agricultural sustainability in India. Furthermore, the Indian Government should integrate development policies in economic and social development and ecological security to increase agricultural sustainability in India.

Also, the values of EEI lie between 0.17-0.82; thus, there exists a significant variation in economic efficiency among the 17 Indian states. Haryana and Punjab have shown the 1st and 2nd positions in economic efficiency. Therefore, these states could create appropriate infrastructure to be in the best position for economic efficiency. Per capita net domestic product, CDR, credit to the agriculture sector by a commercial bank, a ratio of agriculture GDP with the gross sown area, share of irrigated area in gross area sown, a ratio of a net irrigated area with the net sown area, the average size of landholding, a yield of food-grain and oilseed crops, a cropped area under food-grain crops and the ratio of the rural literate population with the gross sown area were positively associated with EEI. Thus, these variables must be considered in other policy formulations to maintain economic development in India. Cash crop farming provides a better economic return to farmers than food-grain farming. Hence, a farming community should grow commercial crops to increase its economic capacity. Consequently, they can apply several inputs to increase their profitability in the agricultural sector. Further, it is suggested that Assam, Bihar, Himachal Pradesh, Odisha, Jammu & Kashmir, Maharashtra, Madhya Pradesh, Uttar Pradesh, Karnataka, Rajasthan, Kerala, Andhra Pradesh, West Bengal, and Gujarat should focus on activities described above to improve their economic efficiency.

The values of SEI lie between 0.241-0.538 across Indian states. Thus, Indian states have high diversity in

social equity due to variations in social development-associated variables. In social equity, Kerala, Himachal Pradesh, and Karnataka have shown the 1st, 2nd and 3rd ranks, respectively. Thus, these states have better performance in social equity among the 17 Indian states. On the other hand, Uttar Pradesh and Bihar were in the lowest position in social equity. SEI was positively correlated with per capita food-grain availability, per capita availability of milk production, total literacy rate, female literacy rate, road length per thousand population, and expenditure on the social sector. Thus, these variables were the most crucial determinants of social equity. Furthermore, social equity decreases with increasing birth rate and infant mortality rates. Therefore, Uttar Pradesh, Bihar, Andhra Pradesh, Madhya Pradesh, Jammu & Kashmir, West Bengal, Odisha, Rajasthan Assam, Gujarat, and Tamil Nadu are suggested to include variables described above in policy formulation to improve their position in terms of social equity. Also, the Indian Government should provide better medical facilities to control infant mortality to increase social development.

Moreover, the values of ESI lie between 0.26-0.72 across Indian states, which proves that these states have high diversity in ecological security. The high diversity in ecological security exists due to variations in available natural resources and ecological services in Indian states. In ESI, Himachal Pradesh and Odisha have shown the 17th and 16th ranks. Therefore, both states performed relatively better in ecological security among the 17 states. Haryana, Tamil Nadu, and Gujarat had the 17th, 16th, and 15th positions in ecological security among the Indian states. The ratio of forest area with the gross sown area, permanent pasture land with the gross sown area, cropping intensity, and annual average precipitation was positively associated with ESI. Forest areas seemed to be the most important factor in mitigating the negative impacts of socio-economic activities and climate change on ecological services. Forest area also works as an ecosystem-adaptation-based approach to mitigate the negative consequences of climate change in the agricultural sector. Thus, the Government should implement a conducive policy to protect the forest area from increasing agricultural sustainability in India. Furthermore, it is also desirable to increase cropping intensity using better irrigation facilities, green technologies, and green fertilizer, conserve traditional crop varieties and develop high-yielding and climate change-resilient varieties of seeds in the agricultural sector to increase ecological security in India. However, a negative impact has been observed between ecological security with a ratio of land not used for cultivation with the gross sown area, fertilizer consumption per hectare land, population density, population growth, and urbanization. Extensive use of fertilizer in cultivation may cause to increase GHGs emissions in the atmosphere. Subsequently, it may be responsible for further escalating climate change and improving environmental degradation. Thus, a farming community should avoid extensive fertilizer use to maintain ecological security. Subsequently, the optimum quantity of cultivated fertilizer will help increase agricultural sustainability. The application of green fertilizer and green and appropriate technology in cultivation may help increase ecological security and agricultural sustainability in India. Furthermore, India should control population density, growth, and urbanization to protect available ecosystem services to increase agricultural sustainability. Overwhelming industrialization is also a main source of GHGs emissions in the atmosphere, which may be caused by increasing the possibility of climate change and environmental degradation. Therefore, Indian Government should focus on green entrepreneurship and green technology to sustain ecological services and sustainable agricultural development.

Empirical results infer that technological advancement in cultivation may help increase agricultural sustainability. Hence, farmers should apply agricultural and green technology to avoid climate change risks in cultivation. Furthermore, it was found that economic efficiency, social equity, and climatic factors have a non-linear association with agricultural sustainability. The estimates also infer that economic efficiency, social equity, and ecological security positively affect agricultural sustainability. Thus, policymakers should centralize an integrated policy to increase economic development, social development, and ecological security. Climatic factors such as AAMaxT, AAMinT, and AARF significantly influence agricultural sustainability. Maximum temperature's impact on agricultural sustainability seemed negative and statistically significant. Thus, agricultural sustainability was adversely affected due to the increased maximum temperature in India. Hence, Indian farmers should apply adaptation strategies to avoid the negative impact of climate shocks on crop farming. For this, India needs to discover alternative options such as technology, heat tolerance crops, irrigation facilities, mixed-cropping pattern, agroforestry, and other practices to mitigate the negative consequences of climate change in agriculture. It was also found that economic efficiency, social equity, and climatic factors have a non-linear association with agricultural sustainability. Existing researchers can also examine the implications of farmers' adaptation strategies, mitigation approaches to climate change, and technological advancement in the agricultural sector in a further study using farm-level information.

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Growth performance and cost-effectiveness of replacement of fishmeal with plant-based protein source, *Leucaena leucocephala* in the diet of *Clarias gariepinus* fingerlings

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Abstract. *Agupugo CS, Nsofor CI, Ezewudo BI, Edeh IC. 2022. Growth performance and cost-effectiveness of replacement of fishmeal with plant-based protein source,* Leucaena leucocephala *in the diet of* Clarias gariepinus *fingerlings. Asian J Agric 6: 28-34.* The present study was conducted to determine the effects of the replacement of fishmeal with *Leucaena leucocephala* (Lam.) de Wit leaf meal (0%, 10%, 20%, and 30%) on fish growth and to compare the cost-effectiveness of replacement of fishmeal with *Leucaena* leaf meal in fish diets. The proximate value of the tested leaf meal showed moderate contents of crude protein and low contents of crude ash. The daily and mean weight gains of fish showed that the highest weight gains were recorded in fish fed with diet T3 (20%), while the least values were in fish fed with diet T4 (30%), and the differences were not significant (P>0.05). The highest survival rate was observed in fish fed with diet T1 (0%), while fish fed with T2 (10%) and T4 had the lowest values. The highest specific growth rate was obtained in diet T1. However, the highest food conversion ratio was recorded in fish fed with diet T4. The highest expenditure was recorded in diet T1. Our findings showed that using *Leucaena* leaf meal in the fish diet is best at a 20% inclusion level for optimum growth. *Leucaena*-containing diets were more cost-effective than a diet with only fishmeal.

Keywords: African mud catfish, cost-benefit, growth indices, leaf meal, nutrient utilization

INTRODUCTION

The aquaculture industry is rising, with an estimated yearly increase of 7% (Chen et al. 2019). Furthermore, for this growth to be sustained, there is a need for the availability of sustainable and economical aquafeeds to fish farmers. Its demands have also increased following the rise in the aquaculture industry (Mensah et al. 2018). Fishmeal, a major protein source in formulated fish diets, has also faced high demand, and it is mainly obtained through capture fisheries from marine and freshwater fish species (Tacon et al. 2006). The recent declines in wild fish stocks, such as the historic collapse of Peruvian anchovies (Ferguson-Cradler 2018), have created an artificial scarcity of fishmeal, culminating in an upsurge in fishmeal cost on fish feed production (Ezewudo et al. 2015). Notably, approximately 10% of the world's fish production is utilized as fishmeal in aquaculture, and this percentage falls short of its high market in fish feed production (FAO 2012). High demand, high cost, and unstable demand and supply emphasize the need to utilize plant proteins as an alternative source of protein to fishmeal in fish diets for sustainable growth of the aquaculture industry.

Leucaena leucocephala (Lam.) de Wit, commonly known as *Leucaena* or white lead tree, is one of the suitable plant protein alternatives to fishmeal for fish feed due to its medium-high protein content, suitable levels of amino acids and, most importantly, very affordable market price tag (De Angelis et al. 2021). However, few studies have demonstrated that *Leucaena* leaf meal can successfully replace fishmeal as a protein source in fish diets at different inclusion levels (Bairagi et al. 2004; Tiamiyu et al. 2015; Babalola and Fakunmoju 2020).

Clarias gariepinus (Burchell, 1822), commonly known as mud catfish or African sharp-tooth catfish, is an omnivorous fish, feeding on fruits, seeds, and varieties of aquatic organisms, including invertebrates, vertebrates, and planktons (Skelton 2001; Odongo et al. 2019). It is widely adopted as a culturable species in Nigeria because of its hardy nature and good feed conversion rate (Sotolu and Faturoti 2011). Fish is in high demand by fish consumers due to the tasty nature of the flesh (Idodo-Umeh 2003). However, a hike in the cost of fishmeal (a major protein source in fish diets) has increased the cost of production, leading to a low supply of fish to consumers. Therefore, this present study was conducted to determine the effects of the partial replacement of fishmeal with plant protein. *Leucaena* leaf meal on growth and nutrient utilization in *C. gariepinus* and compare the cost-effectiveness of the utilization of *Leucaena* leaf meal with that of fishmeal in the diets of *C. gariepinus*.

MATERIALS AND METHODS

Preparation of leaf meal and formulation of experimental diets

Fresh leaves of *L. leucocephala* (Figure 1) planted in Nnamdi Azikiwe University, Awka, Nigeria, were plucked from their branches and taken to the Botany laboratory for identification and authentication (Herbarium No NAUH 206[A]). The plucked leaves were carefully washed and later immersed in clean water for three days to reduce the levels of anti-nutritional elements in most plant proteins. At the end of the three-day immersion, the leaves were sun-dried until they became crispy, and with the aid of a corn milling machine, the leaves were ground into powder. Finally, the milled *Leucaena* leaves were sieved with a hand sieve to obtain fine powder from the milled leaves containing a tiny leaf. The proximate composition of *L. leucocephala* leaves (Table 1) was estimated to determine the total crude protein content, ash, moisture, carbohydrate, fiber, and fat content according to the Association of Official Analytical Chemists (AOAC 2012).

Four experimental diets were formulated according to the protein contents of fish meal, soybean, cornmeal, *Leucaena* leaf meal, and wheat offal by adopting the Pearson Square method (Pearson 1976), as highlighted in Table 2. In addition, *L. leucocephala* leaf meal was partially incorporated in the diets at 0% (the control diet), 10%, 20%, and 30%. The ingredients, such as fishmeal, soybean, cornmeal, etc., purchased from a popular market known as Afor-Nnobi in Idemili South Local Government Area, Anambra State, Nigeria, were used in formulating the diets. First, the formulated diets were weighed, and with the addition of water, the diets were homogenized to give a dough-like paste. Then, with the aid of a 3 mm electronic pelletizer, the diets were pelletized, sun-dried, and packed in airtight plastic containers at 4°C. The formulated diets were later analyzed for proximate compositions (Table 3) following the Association of Official Analytical Chemists (AOAC 2012).

The experimental site, fish, and design

The study was carried out in the Department of Zoology Fish ponds, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. African catfish (*C. gariepinus*) fingerlings were procured from a commercial fish farm in Awka, Awka South Local Government Area, Anambra State, Nigeria. A total of 140 fingerlings with an average weight of 3.6 g and an average length of 8.14 cm were procured and transported in a plastic gallon with well-oxygenated water. The fish were acclimatized for one week in 70 L plastic tanks and fed with commercial fish pellets (Coppens) of 0.8 mm before the commencement of the feeding trial.

At the expiration of the acclimation period, the weights of the remaining fingerlings were obtained electronically (SF-400), and uniform sizes were distributed without being biased in 12 (70 L) plastic tanks for the commencement of the feeding trial. For the feeding trial, 120 fingerlings were used. A 4×3 completely randomized design (CRD) was adopted following the formulation of four (4) dietary inclusion levels of *L. leucocephala* at 0%, 10%, 20%, and 30%. Thirty (30) fingerlings were assigned to each of the treatment diets, and each treatment was replicated thrice such that each replicate had ten fingerlings and placed in a well-netted tank to prevent fish from jumping out. Pipe-borne water was used as the main source of water.

The fish was fed twice daily, between 8.00 am and 6.00 pm. The feeding was at 5% body weight, which was adjusted as they improved in weight. Caution was applied to ensure no left-over feed by siphoning any left-over feed. At the same time, total cleaning of the experimental tanks and the introduction clean water were done twice weekly. The whole research lasted for ten weeks.

Table 1. Proximate values of *Leucaena leucocephala* leaf meal

Parameters	*Leucaena leucocephala* (%)
Crude protein	21.49
Crude fat	3.37
Crude fiber	17.08
Ash	9.88
Moisture	12.34
Dry matter	87.66
Nitrogen free extract	34.85

Table 2. Percentage compositions of *Leucaena leucocephala* leaf meal in the experimental diet

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
Fishmeal (g)	41	37	33	29
Soyabean (g)	27	27	27	27
Corn meal (g)	16	16	16	16
Leucaena leaf meal (g)	0	4	8	12
Wheat offal (g)	10	10	10	10
Methionine (g)	0.25	0.25	0.25	0.25
Lysine (g)	0.25	0.25	0.25	0.25
Starch (g)	2	2	2	2
Salt (g)	0.25	0.25	0.25	0.25
Bonemeal (g)	1	1	1	1
Vitamin premix (g)	0.25	0.25	0.25	0.25
Vegetable oil (g)	2	2	2	2
Total (g)	100	100	100	100
Inclusion levels of *Leucaena* leaf meal (%)	0	10	20	30

Figure 1. *Leucaena leucocephala* leaves with pods

Table 3. Proximate contents of inclusion levels of *Leucaena leucocephala* leaf meal formulated diets

Parameters	T1 (0% L.L.M)	T2 (10% L.L.M)	T3 (20% L.L.M)	T4 (30% L.L.M)
Crude protein	37.17	36.69	37.38	36.78
Crude fat	3.81	3.64	3.76	3.55
Crude fibre	2.11	3.06	2.08	3.14
Ash	7.78	6.94	7.86	6.89
Moisture	8.29	9.11	8.22	9.14
Dry matter	91.71	90.89	91.78	90.86
NFE	37.94	37.56	37.7	46.5

Note: NFE = Nitrogen-free extract. L.L.M = *Leucaena* leaf meal, T1 = (fishmeal as control), T2 = (fishmeal + 10% leaf meal), T3 = (fishmeal + 20% leaf meal), T4 = (fishmeal + 30% leaf meal)

Water quality monitoring

The water temperature was monitored daily with a mercury-in-glass thermometer and recorded to the nearest Celsius (°C). In addition, the pH of the water was taken weekly using a pH meter (Hanna- H198129), and the dissolved oxygen in each experimental tank was determined using YSI dissolved oxygen meter. During the experiment, the water temperature ranged from 26.67-27.79°C, pH 6.28-6.48, and dissolved oxygen 3.95-4.27.

Determination of growth and feed utilization of fish

The following growth and feed utilization indices were computed before and after the feeding trial on each diet following the formulae reported in Ezewudo et al. (2015).

$$\text{Daily weight gain (g/fish)} = \frac{FW - IW}{7 \text{ days}}$$

Where FW = Final weight (g/fish) and IW = Initial weight (g/fish)

Mean weight gain (g/fish) = FMW – IMW

Where IMW = Initial mean weight (g/fish) and FMW = Final mean weight (g/fish)

Mean Length gain (cm/fish) = FML – IML

Where IML = Initial mean length (cm/fish) and FML = Final mean length (cm/fish).

$$\text{Specific growth rate} = \frac{\log_e FMW - IMW}{T} \times 100$$

Where $\log_e$ = natural logarithm; IMW = initial mean weight (g/fish); FMW = final mean weight (g) and T = total duration of the experiment

$$\text{Relative growth rate (RGR)\%} = \frac{FW - IW}{IW} \times 100$$

Where IMW = Initial mean weight (g/fish) and FMW = Final mean weight (g/fish)

$$\text{Survival rate (\%)} = \frac{Nf \times 100}{Ni}$$

Where Ni = Number of fish at the beginning of the experiment and Nf = Number of fish at the end of the experiment

$$\text{Food conversion ratio (FCR)\%} = \frac{\text{Total food fed to fish (g)}}{\text{Total weight gain by fish (g)}}$$

Food intake is the amount of food fed to the fish – food left-over; this is done daily by siphoning the left-over food, drying and reweighing them to ascertain the quantity eaten by fish.

Cost-benefit analysis of the production of experimental feed

Cost per kilogram of feed types = Quantity of each ingredient × cost of 1 kg of the ingredient/quantity of feed formulated (1000 g).

Cost of feed consumed per fish = Total food consumed per fish × feed cost per kilogram.

Expenditure per fish = Fish-cost of 1kg in the market + cost of food consumed by the fish.

Statistical analysis

All data obtained were subjected to one-way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS), Version 23 for Windows. Differences in means were separated using Duncan's new multiple-range test. The significant difference was established at a 5% probability level ($P<0.05$), while the results generated were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Determination of growth and feed utilization of fish

The daily and mean weight gains of fish fed with different inclusion levels of *L. leucocephala* leaf meal showed that all the fish in each treatment recorded progressive weight gains (Table 4). The highest daily and mean weight gains were recorded in fish fed with diet T3 containing 20% inclusion levels of *Leucaena* leaf meal (4.3±1.127 g and 30.10±7.894 g), while most minor increases were observed in those fed with diet T4 (3.23±0.587 g and 22.60±4.355 g). There was no significant difference ($P>0.05$) in weight gains of *C. gariepinus* fingerlings fed with the different experimental diets.

There was a progressive increase in the weekly length increase of *C. gariepinus* fed varying inclusion levels of *L. leucocephala* leaf meal for ten weeks (Table 4). The highest mean length increase (9.12±1.41 cm) was recorded in fish fed with diet T, while those fed with diet T4 recorded the least mean length increase (8.14±0.70 cm) (Table 4). The analysis of variance result revealed no significant difference ($P>0.05$) between the mean length

gains of *C. gariepinus* fed varying inclusion levels of *Leucaena* leaf meal.

Data from the specific growth rate of *C. gariepinus* fingerlings fed with varying concentrations of *L. leucocephala* feed meal revealed that the highest specific growth rate was obtained in diet T1 (4.85±0.267%). In contrast, the lowest was generated in those fed with diet T4 (4.44±0.272%), and the differences were not significant (P>0.05) (Table 4). Furthermore, the highest relative growth rate (872.57±181.502%) was obtained in fish-fed diet T1 (control diet) while the least value (647.76±134.433%) was recorded in those fed with diet T4 and the differences were non-significant (P>0.05) (Table 4).

Mortality was observed during the feeding trial. However, the highest survival rate (96.66±1.93%) was recorded in diet T1 (control diet), while the lowest values (90.0±5.77%) were observed in fish fed with diets T2 and T4, and the differences were significant (P<0.05) (Table 4).

The results of total feed consumed by *C. gariepinus* fingerlings fed with the four experimental diets for ten weeks revealed that the mean feed intake of *C. gariepinus* fingerlings was highest (7.10±0.319 g) in those fed with the diet T3, while the lowest value was obtained in those fed with diet T4 (6.28±0.887 g). Differences were significant (P<0.05) (Table 5). In addition, the highest feed conversion ratio was recorded in *C. gariepinus* fingerlings fed with the diet T4 (0.28±0.014%), while the least value (0.23±0.01855%) was recorded in those fed with diet T1 and the differences were not significant (P>0.05) (Table 5).

Cost of production of the four feed types for profitable maintenance of aquaculture

Upon the completion of the feeding trial, cost-benefit production of fish (*C. gariepinus*) fingerlings fed four dietary treatments of *Leucaena* leaf meal were compared using the following indices: cost/kg, cost of total feed consumed, and expenditure (Table 6). The highest cost/kg, total feed consumed, and expenditure was recorded in diet T1, followed by diet T2, and the lowest values were obtained in diet T4 (Table 6).

Discussion

The appreciable contents of crude protein, crude fats, crude fiber, and ash in *Leucaena* leaf meal suggest that this leaf in animal diets can provide the required proteins, minerals, dietary fiber, and essential fatty acids needed for animal metabolism as efficient growth and improve food digestibility. The crude protein of the tested leaf meal of 21.49% compares well to the 21.49-22.29% reported by De Angelis et al. (2021). However, the present crude protein content is far from the 22.67-29.17% recorded by Adekojo et al. (2014). The variations in crude protein content of *Leucaena* leaf meal, as reported by different researchers, could be attributed to the nutritional constituents of the soil on which the plant was grown, the age of cultivars, and the processing methods deployed before proximate composition analysis of the leaf meal (Ayssiwede et al. 2010; Adekojo et al. 2014; Figueredo et al. 2019). According to Adekojo et al. (2014), variations in proximate compositions of *Leucaena* leaf meal depend on the different processing methods, namely air-drying; soaking in fresh water at room temperature for 36 hours; soaking in hot water for 24 hours and fermenting for five days.

Table 5. Effect of partial replacement of fishmeal with four levels of *Leucaena leucocephala* leaf meal on feed utilization of *Clarias gariepinus*

Treatments	Mean feed intake (g)	Mean FCR (%)
T1: Control	6.82±0.746^{b}	0.23±0.018
T2: 10% *Leucaena* leaf meal	6.98±0.232^{b}	0.26±0.009
T3: 20% *Leucaena* leaf meal	7.10±0.319^{b}	0.24±0.052
T4: 30% *Leucaena* leaf meal	6.28±0.887^{a}	0.28±0.014

Note: Different letters in one column mean significant differences at P<0.05. Absent of letters means no significant differences between treatments

Table 6. Cost-benefit production of fish (*Clarias gariepinus*) fingerlings fed four dietary treatments of *Leucaena leucocephala* leaf meal

Parameters	T1	T2	T3	T4
Cost of 1 kg fish ($)	2.92	2.92	2.92	2.92
Mean initial weight (g)	3.47	3.83	3.63	3.50
Mean final weight gain (g)	30.07	26.70	30.10	22.60
Cost/kg feed ($)	5.60	5.10	4.63	4.16
Cost of total feed consumed ($)	0.37	0.35	0.32	0.27
Expenditure ($)	3.29	3.27	3.24	3.19

Note: T1 = (Fishmeal as control), T2 = (Fishmeal and 10% leafmeal), T3 = (Fishmeal and 20% leafmeal) and T4 = (Fishmeal and 30% leafmeal)

Table 4. Effect of partial replacement of fishmeal with four levels of *Leucaena leucocephala* leaf meal on growth performance of *Clarias gariepinus*

Treatments	Daily weight gain (g)	Mean weight gain (g)	Mean length gain (cm)	Specific growth rate (%)	Relative growth rate (%)	Survival (%)
T1: Control	4.29±0.715	30.07±5.314	9.12±1.41	4.85±0.267	872.57±181.502	96.66±1.93^{c}
T2: 10% *Leucaena* leaf meal	3.81±0.108	26.70±0.872	8.83±0.48	4.69±0.047	698.99±48.065	90.0±5.77^{a}
T3: 20% *Leucaena* leaf meal	4.3±1.127	30.10±7.894	8.97±1.83	4.83±0.371	826.45±203.756	93.33±3.58^{b}
T4: 30% *Leucaena* leaf meal	3.23±0.587	22.60±4.355	8.14±0.70	4.44±0.272	647.76±134.433	90.0±5.77^{a}

Note: Different letters in one column mean significant differences at P<0.05. Absent of letters means no significant differences between treatments

The crude fat content of the present study was lower than the 5.65% reported by Malik et al. (2019). Malik et al. (2019) further reported that the seeds of *L. leucocephala* contain more fats than their leaves; however, the latter is richer in nutritional fats, especially polyunsaturated fatty acids, than saturated fatty acids. The crude fat present in *Leucaena* leaf meal was observed when *Leucaena* leaf meal was used in replacing a commercial broiler finisher diet in the diets of black australorp and Potchefstroom koekoek chicken (Thamaga et al. 2021). These authors reported an increase in the levels of crude fats in diets with *Leucaena* leaf meal compared with the control diet (0% *Leucaena* leaf meal). The moderate ash level in the tested leaf meal shows that it is well endowed with minerals. Thamaga et al. (2021) showed that the *Leucaena* leaf meal is rich in essential minerals like copper, manganese, zinc, and iron but contains a lesser amount of calcium, magnesium, potassium, and sodium.

The crude fiber reported in this current study was higher than the 13.85% reported by Adedeji et al. (2013) but lower than the 19.20% reported in the work of Babalola and Fakunmoju (2020). The improved crude fiber in the tested leaf meal can aid bowel movement, favoring nutrient absorption and reducing constipation (Lunn and Buttriss, 2007; Amobi et al. 2019).

The proximate contents of the experimental diets showed that they were rightly formulated to provide the necessary nutrient for optimum fish growth. The crude protein of *Leucaena* leaf meal-reinforced diets (36.69-37.38) is quite close to 35.08-36.81 observed by Tiamiyu et al. (2015). The results from the present study on crude protein (CP) and ash showed that the highest CP and ash were recorded in diet T3 with 20% *Leucaena* leaf meal against the control diet with 0% *Leucaena* leaf meal. This increase indicates that better right contents of crude protein and minerals could be achieved at a 20% inclusion level against the other concentration levels (0%, 10%, and 30%). However, despite the moderate contents of crude protein and minerals in the *Leucaena* leaf meal, it was observed that the inclusion of *Leucaena* leaf meal above 20% recorded a decrease in crude protein and ash. The processing method adopted for the Leucaena leaf meal could better explain this result. According to Adekojo et al. (2014), the sun-drying method used in this study contains more anti-nutritional elements like mimosine, capable of removing essential nutrients, unlike other processing methods like immersion in freshwater, soaking in hot water and fermentation.

High fiber content was recorded in the diet with a 30% inclusion level of *Leucaena* leaf meal against the control, while the crude fat was highest in diet T1 with 0% *Leucaena* leaf meal. This result is not surprising since the *Leucaena* leaf meal is enriched with fiber (Malik et al. 2019), while the high crude fat recorded in diet T1 could be attributed to high-fat contents present in animals than in plants (Nnamonu et al. 2020).

Fish is one of the animals known to adapt to various nutritional states. The ability of fish to accept, utilize and convert the food given to it for optimum growth and productivity is best studied using growth and feed utilization indices (González-Rodríguez et al. 2014; Chen et al. 2019). Fingerlings fed with a 20% inclusion level of *Leucaena* leaf meal (diet T3) had the best daily and mean weight gains. That could be attributed to the highest crude protein and ash recorded in this diet and elevated level of crude fat, which supports anabolic processes like growth and deposition of fat. The highest weight gain recorded in fish fed with a 20% inclusion level of the *Leucaena* leaf meal can also be associated with the high acceptability and palatability of the feed. Moreover, fish fed this diet had the highest mean feed intake than those fed with varying concentrations of the tested leaf meal. This finding agrees with Amisah et al. (2009) and Tiamiyu et al. (2015). These authors reported that the inclusion level of *Leucaena* leaf meal at 20% did not negatively alter the weight of fish but instead gave the best weight gain. The best specific growth rate and mean length gain recorded in fish fed with a control diet may be attributed to high amino acids present in fishmeal than in plant proteins. Schulz et al. (2007) opined that incorporating high levels of plant proteins against the conventional fishmeal in fish diets is associated with retarded growth performance. Furthermore, according to Reigh (2008), plant proteins possess lower amino acid profiles than animal proteins like fishmeal, which are already replaced.

High survival rates recorded in this study could be due to the proper handling of the fish and proper water quality management. However, fish fed with diets T1 and T3 had the best survival rates, indicating the suitability of these diets for fish. According to Tiamiyu et al. (2015), high fish survival rates in experimental trials are good indicators of the proper handling of fish, the suitability of the diets for fish, and good water quality management.

Growth in animals does not only manifest when the right food is given but also depends on the ability of the animal to efficiently convert the food given into tissues and muscles for optimum growth (Olivotto et al. 2003). The lowest food conversion ratios recorded in diets T3 and T1 indicate that fish fed with these two diets did not effectively convert their food to body growth than those fed with diet T4, which had the highest food conversion ratio (Fry et al. 2018). The lowest food conversion ratios recorded in diets T3 and T1 could be likened to low crude fiber, elevated crude protein, and low levels of anti-nutritional elements, which promote or support food digestibility (Hermawan et al. 2021). On the other hand, diet T4, with 30% inclusion levels of *Leucaena* leaf meal which recorded the highest food conversion ratio, could be attributed to high fiber contents in the diet due to the high inclusion level of plant protein (*Leucaena* leaf meal), low crude protein and high levels of nutrient-inhibitory elements like saponin and mimosine leading to poor digestibility and palatability (Agbo et al. 2011).

The cost-benefit production of fish (*C. gariepinus*) fingerlings fed four varying concentrations of *Leucaena* leaf meal showed that the cost per kilogram of feed types, cost of feed consumed per fish, and expenditure per fish decreased with an increase in inclusion levels of *Leucaena* leaf meal. That was due to the high cost of the fish meal, which was higher in the control diet than the three

remaining diets containing varying concentrations of *Leucaena* leaf meal. The cost-effectiveness assessment of the current work clearly showed that *Leucaena*-reinforced diets are cheaper than the control diet; however, the 20% inclusion level of *Leucaena* leaf meal was more profitable with the best weight gain than the other remaining diets. This finding suggests that more monetary profits and better productivity await a fish farmer when 20% of *the L. leucocephala* leaf meal is incorporated into the fish diet to replace fishmeal. This result agrees with the finding of Agbo et al. (2011), who reported more profit in fish diets incorporated with cottonseed meal than in the control diet with only fishmeal.

In conclusion, findings from this present study indicated that the *Leucaena* leaf meal is highly nutritious and can be incorporated into animal diets, including fish as a feed ingredient. However, its utilization in fish diets is best at a 20% inclusion level for optimum growth and efficient feed utilization. In contrast, higher incorporation of the leaf meal in fish diets could retard fish growth due to high fiber contents and anti-nutritional elements in the leaf meal. Additionally, diets containing *Leucaena* leaf meals were more cost-effective than those with fish, especially at a 20% inclusion level of *Leucaena* leaf meal. Based on the current findings, we suggest replacing fishmeal with *Leucaena* leaf meal at a 20% inclusion level be adopted in fish diet formulation for more monetary profits and better productivity. Future research should focus on evaluating the potency of *L. leucocephala* seeds as feed ingredients in fish diets and compare their growth performance to those fed with *L. leucocephala* leaf meal.

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Effect of vermicompost and biostarter on the growth and photosynthetic rate of *Echinacea purpurea*

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Abstract. *Choirunnisa LF, Solichatun, Yunus A. 2022. Effect of vermicompost and biostarter on the growth and photosynthetic rate of* Echinacea purpurea*. Asian J Agric 6: 35-39. Echinacea purpurea* (L.) Moench, or purple coneflower, is a medicinal plant that originated in North America and began to cultivate in Indonesia. A proper method is needed to improve its growth and development to adjust and cultivate in tropical areas like Indonesia. This study aimed to determine the effect of vermicompost and biostarter on the growth and photosynthetic rate of *E. purpurea.* Therefore, Split-Plot Randomized Complete Block Design was used. Two factors of this study were organic materials such as vermicompost and biostarter. Both can increase plant growth and the photosynthetic rate of *E. purpurea.* The dosages of vermicompost were 0, 40, 60, and 80 g/plant, and different types of biostarter from Banana peel waste and EM. This study also investigated data that included plant height and width, leaf numbers, leaf area, photosynthetic and transpiration rate, and stomata conductance. The study showed that the treatment of 80 g/plant vermicomposts and EM highest resulted in plant height (73,6 cm), leaf numbers (82), and stomatal conductance (0,4585 mol $m^{-2}s^{-1}$). Moreover, the leaf area (87,21 cm^2) and photosynthetic rate (0,6839 µmol $m^{-2}s^{-1}$) showed the highest result with the treatment of 80 g/plant vermicomposts and biostarter from Banana peel waste. On the other hand, the treatment of 60 g/plant vermicompost showed the best result on plant width (50,25 cm) also transpiration rate (0,2390 mmol $m^{-2}s^{-1}$). This study concluded a significant effect between vermicompost and biostarter on the growth and photosynthetic rate of *E. purpurea*.

Keywords: Biostarter, *Echinacea purpurea,* growth, photosynthetic rate, vermicompost

INTRODUCTION

Echinacea purpurea (L.) Moench, or purple coneflower, is a medical plant that originated in North America and began to cultivate in Indonesia. This plant is widely cultivated as medicinal because it increases the human immune system. Many species of *Echinacea* besides *E. purpurea*, like *E. angustifolia* and *E. pallida*, have been identified as important medicinal plants. The morphology characteristics of *E. purpurea* turned out to have various changes after being developed and cultivated in Indonesia. The clear morphological differences are flowers. According to Siddiq et al. (2020), the form of *E. purpurea* flowers cultivated in the lowland area has changed, which correlated with its parent. The shape of the corolla is curved downward, and some are horizontal.

Organic fertilizer is very important in supporting plant growth and development because it provides essential nutrients needed by plants. Vermicompost is an organic fertilizer that uses a worm to convert residues into a secondary organic fertilizer. This fertilizer can improve soil fertility by increasing the nutrients of the soil. In addition, vermicompost can increase water availability and mineral nutrients (Blouin et al. 2019).

Furthermore, it has been reported that using vermicompost significantly increases the level of photosynthetic pigments such as chlorophyll a&b, total chlorophyll, and carotenoids by increasing nitrogen content in plants. It is possible because the fertilizer can improve the soil's structure, increase humidity, and supply nutrients to the plant. The application of vermicompost can increase the level of photosynthetic pigments, sunlight absorption capacity, the material production of photosynthesis, and plant growth and development (Afkari 2018; Jaikishun et al. 2018).

Biostarter is a liquid that contains various microorganism decomposers and can be useful in decomposing organic waste. Biostarter can be made by utilizing organic waste after fermentation for approximately 7-14 days (Sukmawati et al. 2019). Waste fruit peels are alternative raw materials for biostarter because they contain various nutrients such as carbohydrates, glucose, sodium, potassium, and phosphorus that are suitable for them (Widyabudiningsih et al. 2021). Another type of biostarter used in this study is "Effective Microorganism" (EM), a mixture of various beneficial microorganisms for plant growth. EM can be used to improve the decomposition of organic waste,

increase the plant's nutrients, and suppress the activity of pathogenic microorganisms (Joshi et al. 2019).

Organic materials such as vermicompost and biostarter can increase plant growth and the photosynthetic rate of *E. purpurea.* However, since *E. purpurea* is an introduced species from a subtropical country, a proper method is needed for improving its growth and development to adjust and cultivate in a tropical area like Indonesia.

The study aimed to determine the effect of vermicompost and biostarter (Banana peel waste and EM) on the growth, and photosynthetic rate of *E. purpurea* cultivated in lowland area (± 300 meters above mean sea level), Experimental Garden Faculty of Agriculture, Universitas Sebelas Maret, Sukosari, Jumantono, Karanganyar, Central Java, Indonesia.

MATERIALS AND METHODS

Experimental area and soil properties

This open-field study was conducted from July to November 2021 and is located in a lowland area (± 300 meters above mean sea level), Experimental Garden Faculty of Agriculture, Universitas Sebelas Maret, Jumantono, Karanganyar, Central Java, Indonesia (7º37'829" S, 110º56'901" W). The temperature was captured between 27 to 33^{0}C, and the humidity was around 50-60%. The type of soil used in this experimental garden identified as alfisols, with the characteristics is shown in (Table 1).

Procedures

Collection of seeds and preparation of seedlings

Echinacea purpurea seeds were chosen from the Research Center of Medical Plants and Traditional Medicines (B2P2TOOT) collection, Tawangmangu, Karanganyar, Central Java, Indonesia. First, the dried flowers of *E. purpurea* were sorted to select the best seeds from the flower with an embryo. Then, the dry seeds were planted to grow a seedling in the pot tray until the age of six weeks and transplanted into the experimental garden.

Planting and maintenance

The seedlings were planted in the experimental garden with a Split-Plot Randomized Complete Block Design with two factors and three replications. Vermicompost dosages (0, 40, 60, 80 g/plant) and different types of biostarter from Banana peel waste and effective microorganisms with the trademark "EM4". In control plots, neither vermicompost nor biostarter was applied. So, there were 12 combinations of treatments repeated three times with a total of 270 plants of *E. purpurea.* The plant's watering was done every day (morning or afternoon) with the sprinkler irrigation system. Weed control was done manually every week. This current study was conducted in the 2021 rainy season.

Observation

Plant height and width, leaf numbers and area were observed when the plants were six weeks after transplanting in the experimental garden until the flowers bloomed. Measurements were made on 15 plants for each parcel, or 60% (180 plants) were used to determine agronomic performance. In addition, photosynthetic, transpiration rate, and stomatal conductance were observed with the natural light, from 8.30 AM to 11.30 AM, with a Plant photosynthesis meter NY-1020 (Zhengzhou Nanbei Instrument Equipment Co., Ltd.).

The leaf area measurement was done with non-destructive models by a combination of leaf length and maximum width. Based on Aminifard et al. (2016), leaf area estimation on *E. purpurea* can be done by measuring leaf dimension and resulting in the most accuracy with the formulation [LA = 0,575 (Length x width) – 0,934]. A ruler measured the length and width of the leaves.

Data analysis

The results were analyzed with Two Way Analysis of Variance (ANOVA) and continued with Duncan Multiple Range Test (DMRT) at the 5% level to know the significant effect of the treatment. The data were analyzed with SPSS 20.0 version.

RESULTS AND DISCUSSION

Of the total of 270 E. purpurea, 60% were used as a sample (180 plants). The growth rate parameters are height, width, leaf numbers and area. The photosynthetic rate parameters are photosynthetic rate, transpiration rate, also stomata conductance.

Plant height

Plant height is one of the plant growth parameters and indicates the plant's physiological function. Therefore, this measurement is frequently used to know the effect of the treatments given in the study. The plant height measured is the distance of the upper boundary of the plant or the upper part above the ground.

Quantitative analysis shows that the height of *E. purpurea* among the treatments given is significantly different in DMRT at a 5% level. The results showed that the treatment of 80 g/plant vermicomposts and EM had the highest result in plant height (73,6 cm) compared to the control with only 23,60 cm (Table 2). The addition of vermicompost has a positive effect on plant growth and development. Its also been reported that nitrogen, phosphorus, potassium, several micronutrients, and microbial and enzymatic activities were correlated to it. The use of vermicompost enhanced the quality of the plant by increasing its nutritional status (Awadhpersad et al. 2021).

Table 1. Soil properties in the experiment

Characteristic	N (%)	P_2O_5 (%)	K_2O (%)	Mg (%)	pH	C organic (%)
Vermicompost	1.70	1.10	1.49	0.26	7.6	15.47

Table 2. Effect of vermicompost and biostarter on plant height (cm) of *Echinacea purpurea*

Treatments	Vermicompost dosages (g/plant)			
biostarter type	0	40	60	80
Control	23.60c	30.80c	35.45c	58.00c
Banana peel waste	43.20b	46.20ab	47.00b	65.90b
EM	44.60a	56.60a	65.20a	73.60a

Note: Numbers followed by different letters in the same column show a significant difference in DMRT at a 5% level

Plant width

The important thing to measure besides plant height is plant width. Plant width is an essential geometric trait among various phenotypes that can indicate plant growth rate. The plant width we measured is the canopy width or distance between one edge of the leaf on a plant to the other. Based on Table 3, the treatment of 60 g/plant vermicomposts EM showed the best result on plant width (50,25 cm) compared to the control (42,20 cm). El-Mageed et al. (2020) reported that EM could help plants increase nutrient uptake and enhance plant growth. On the other hand, EM can activate the beneficial microorganisms in the soil and improve the soil's physical and chemical characteristics.

EM is a solution containing various beneficial aerobic and anaerobic, nonpathogenic microorganisms considered a soil starter or activator improving soil structure, fertility, and nutrient cycling (Talaat et al. 2015).

Leaf numbers

The treatment of 80 g/plant vermicomposts and EM gave the highest result on leaf numbers (82,60) compared to the control (45,40). The results are given in Table 4. Using vermicompost can increase plant growth properties like leaf number. That can be attributed to plant growth regulators such as auxin. Ahmadpour and Armand (2020) stated that Zinc (Zn) is an important element from vermicompost and plays an important role in the structure of Tryptophan Amino Acid (the main precursor to the auxin synthesis). Auxin can increase cell walls, leading to an increase in the longitudinal growth of the plant.

Organic fertilizers like vermicompost play an important role in the plant growth regulator (as auxin and cytokinin) by increasing the microorganism community and soil activity. Also, humid acid found in the vermicompost has many elements that increase the plants' availability and improve their growth and development properties (Amiri et al. 2017).

Leaf area

Leaf area is a very important growth parameter and effective monitoring for the growth and development of the plant. Leaf area showed the highest result with the treatment of 80 g/plant vermicomposts and biostarter from Banana peel waste (87,21 cm^2) compared to the control is 59,34 cm^2 (Table 5).

According to Ahmadpour and Armand (2020), increasing the leaf area shows more photosynthetic capacity. There is a direct relationship between the photosynthetic system to dry matter yield. The addition of vermicompost has a role in maintaining water-soluble nutrients from the root to the leaf by passive transmission in the xylem. The macro and microelements of the vermicompost also nourish the leaf and leaf morphology.

Photosynthetic rate

Photosynthetic rate is one of the photosynthetic parameters determined by the portable photosynthesis meter NY-1020 (Zhengzhou Nanbei Instrument Equipment Co., Ltd.). Table 6 shows the treatment of 80 g/plant vermicomposts and Banana peel waste resulted in a photosynthetic rate (0,6839 µmol m^{-2} s^{-1}) compared to the control (0,0931 µmol m^{-2} s^{-1}). Conversely, the photosynthetic reduction rate in the treatment of 0, 40 g/plant vermicomposts and Banana peel waste from 0,4237 µmol m^{-2} s^{-1} to 0,1117 µmol m^{-2} s^{-1} is still unresolved. Mu and Chen (2021) stated that the decreasing photosynthesis rate might be the physiological response to N deficiency and allocation in leaf structure.

Ahmadpour and Armand (2020) reported that adding biofertilizers like vermicompost increases the microorganism's activity in the soil and has a main role in nitrogen fixation. This terrestrial microorganism's activity leads to releasing the plants' required elements (zinc, iron, manganese, and magnesium) that have the main role in chlorophyll structure. Therefore, increasing the macronutrients (Nitrogen, Phosphorus, Potassium, Calcium, and Magnesium) and micronutrients (Iron, Zinc, Copper, and Manganese) in the leaf is important to the photosynthetic. Moreover, vermicompost also has the primary role in maintaining the photosynthetic system and pigments stability.

Table 3. Effect of vermicompost and biostarter on plant width (cm) of *Echinacea purpurea*

Treatments	Vermicompost dosages (g/plant)			
biostarter type	0	40	60	80
Control	42.20b	42.40b	45.20b	47.45b
Banana peel waste	45.00a	46.20a	49.80a	50.20a
EM	45.80a	47.80a	50.25a	48.00a

Note: Numbers followed by different letters in the same column show a significant difference in DMRT at a 5% level

Table 4. Effect of vermicompost and biostarter on leaf numbers of *Echinacea purpurea*

Treatments	Vermicompost dosages (g/plant)			
biostarter type	0	40	60	80
Control	45.40b	46.33b	47.75b	54.50b
Banana peel waste	55.20a	70.80a	74.00a	68.40a
EM	63.20a	72.60ab	75.20a	82.60a

Note: Numbers followed by different letters in the same column show a significant difference in DMRT at a 5% level

Table 5. Effect of vermicompost and biostarter on leaf area (cm^2) of *Echinacea purpurea*

Treatments	Vermicompost dosages (g/plant)			
biostarter type	0	40	60	80
Control	59.34a	69.80ab	70.39b	79.67c
Banana peel waste	65.21a	64.42ab	72.82b	87.21c
EM	67.02a	69.84ab	78.93b	83.83c

Note: Numbers followed by different letters in the same column show a significant difference in DMRT at a 5% level

Table 6. Effect of vermicompost and biostarter on photosynthetic rate (µmol $m^{-2}s^{-1}$) of *Echinacea purpurea*

Treatments	Vermicompost dosages (g/plant)			
biostarter type	0	40	60	80
Control	0.0931b	0.1741b	0.2708ab	0.5332a
Banana peel waste	0.4237b	0.1117b	0.4096ab	0.6839a
EM	0.2234b	0.2864b	0.6486ab	0.4865a

Note: Numbers followed by different letters in the same column show a significant difference in DMRT at a 5% level

Table 7. Effect of vermicompost and biostarter on transpiration rate (mmol $m^{-2}s^{-1}$) of *Echinacea purpurea*

Treatments	Vermicompost dosages (g/plant)			
biostarter type	0	40	60	80
Control	0.0323	0.0754	0.2390	0.0880
Banana peel waste	0.0795	0.1548	0.0691	0.1185
EM	0.1873	0.0596	0.1873	0.1249

Note: Numbers followed by different letters in the same column show a significant difference in DMRT at a 5% level

Table 8. Effect of vermicompost and biostarter on stomatal conductance (mol $m^{-2}s^{-1}$) of *Echinacea purpurea*

Treatments	Vermicompost dosages (g/plant)			
biostarter type	0	40	60	80
Control	0.0405c	0.2848bc	0.2843ab	0.3407a
Banana peel waste	0.2456c	0.2720bc	0.3689ab	0.4356a
EM	0.1196c	0.1843bc	0.4574ab	0.4585a

Note: Numbers followed by different letters in the same column show a significant difference in DMRT at a 5% level

The addition of vermicompost also increases the stability of the photosynthetic system. Maintaining the process of photosynthesis depends on how the plant produces and receives the high-energy molecules, including Adenosine Triphosphate (ATP) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH), by taking the sunlight energy. The addition of vermicompost increases CO_2 production in the soil and increases microorganism activity. The better production of CO_2 in the root environment plays an important role in providing CO_2 photosynthesis (Hosseinzadeh et al. 2018).

Transpiration rate

Transpiration rate is the gas exchange parameter and is directly related to photosynthesis. The treatment of 60 g/plant vermicompost with no biostarter showed the best result on transpiration rate (0,2390 mmol m^{-2} s^{-1}) compared to the control is 0,0323 mmol m^{-2} s^{-1} (Table 7). However, in the transpiration rate, we found no significant effect on adding vermicompost and biostarter. Therefore, the results on leaf transpiration are unclear between the treatments and control. Still, Pereira et al. (2021) stated that it might be related to the presence of phytohormones in its plant. Also, the reduction level of transpiration rate resulted in higher water use efficiency and CO_2 assimilation.

Stomatal conductance

The treatment of 80 g/plant vermicomposts and EM highest resulted in stomatal conductance (0,4585 mol m-2s-1) compared to the control, which is 0,0405 mol m^2s^{-1} (Table 8). It has been reported that the higher result of stomatal conductance between the treatments and the control could be attributed to their higher water-holding capacity, which reduced the water stress level (Mahmud et al. 2020). Stomatal conductance regulates the uptake of CO_2 photosynthesis and water loss through transpiration. The result of stomatal conductance observed in the field was rarely at the absolute maximum or achievable when the specific growth conditions were at the optimum condition. The measurement was on interveinal areolae at the mid-surface of the healthy, fully expanded, and sun-exposed leaves (Murray et al. 2019). Environmental factors affect stomatal conductance, including water and nutrient status, light, CO2 levels, and temperature. As the main nitrogen source for plants, nitrate could regulate stomatal movement (Mu and Chen 2021).

This study concluded that there is a significant effect between vermicompost and biostarter on the growth and photosynthetic rate of *E. purpurea*. The treatment of 80 g/plant of vermicompost and EM showed the best result on plant height, leaf numbers, and stomatal conductance. Furthermore, the treatment of 80 g/plant of vermicompost and biostarter from Banana peel waste showed the highest leaf area and photosynthetic rate. The treatment of 60 g/plant vermicompost showed the best result on plant width also transpiration rate. Further studies are necessary to promote the integration of vermicompost and biostarter in *E. purpurea* to improve its growth and development to adjust and cultivate in a tropical area like Indonesia. A good adaptation for an introduced species like *E. purpurea*, especially in Indonesia, is very important because this plant is quite a promising opportunity to develop medicinal uses and products.

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Assessment of pests, natural enemies and soil microorganisms in lowland rice field under organic and inorganic production systems

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Abstract. *Dela Peña WB, Ratilla BC. 2022. Assessment of pests, natural enemies and soil microorganisms in lowland rice fields under organic and inorganic production systems. Asian J Agric 6: 40-46.* Farmers readily use synthetic pesticides over organic and natural pest management strategies in controlling pests that may disrupt the ecological balance. This study assessed the population of insect pests, natural enemies, and soil microorganisms associated with lowland rice PSB Rc18 grown under different production systems. A lowland area at the Department of Agronomy, Visayas State University, Visca, Baybay City, Leyte, Philippines, was used to produce organic rice for four consecutive croppings. Results revealed that organic farmers' practice in Leyte (T2) showed a higher population of natural enemies, especially mirid bugs, fewer brown planthoppers, and green leafhoppers at 14-44 DAT and the number of folded leaves observed. However, conventional farmers' practices in Leyte (T3) had the highest incidence and severity of bacterial blight and rice blasts. On the other hand, bacterial and fungal microorganisms were found to be associated with the soil samples. Furthermore, the fungal population increased in both organic production systems compared to the conventional production system. Hence, organic production systems increased the number of beneficial insects and soil microbes' populations that may, directly and indirectly, affect pests and diseases in lowland rice.

Keywords: Lowland rice, microbial population, natural enemies, organic production system

INTRODUCTION

Lowland rice fields are considered a unique and varied ecosystem. It is characterized by rapid physical, chemical, and biological changes and has a large diversity of floral, faunal, and microbial species. Most of these species are beneficial, such as predators, parasitoids, and soil microorganisms (MEA 2005; Acosta et al. 2016). On the other hand, it is also home to several insect pests and diseases. Accordingly, about 44 diseases causing microorganisms (Hollier 1994) and about 187 species of insect pests (Yunus and Ho 1980) in rice are known to cause greater economic losses. It is estimated that the global losses in rice due to weeds, animal pests, and diseases reach 10.2%, 15.1%, and 12.2%, respectively, of the total rice production per year (Oerke 2006).

Studies have shown that organic crops are more resistant to pest attacks due to their thicker cell wall and lower free amino acid levels than conventional rice (Ramesh et al. 2005). Also, the presence of natural enemies is enhanced under organic systems, thus reducing the pest population (Hesler et al. 1993; Drinkwater et al. 1995). In addition, some insect species (lady beetles, ground beetle, crickets, long-horned grasshopper, water bugs, and damselfly) and spiders often control insect pests and maintain a balance insect population (Shepard et al. 1987. For instance, the hymenopteran parasitoids, *Telenomus dignus*, *Tetrastichus schoenobii*, and *Trichogramma japonicum* parasitize 75.29 to 97.56% of stem borer eggs (Rama et al. 2013). Jayakumar and Sankari (2010) also reported five spiders, namely: *Lycosa pseudoannulata*, *Callitrichia formosana*, *Tetragnatha javanas*, *Argiope catenulata*, and unidentified *Plexippus* species, that successfully reduced six different insect pests, such as *Nephotettix virescens*, *Scirpophaga incertulas*, *Cofana spectra*, *Cnaphalocrosis medinalis*, *Nilaparvata lugens*, and *Leptocorisa acuta*.

However, the production practices affect the ecological balance of different organisms within the rice ecosystem. With the extent to achieve the targeted potential yield of modern varieties nowadays, intensive crop management practices were introduced (Byerlee 1994). The higher application of synthetic fertilizers and periodic pesticide spraying resulted in environmental toxicity, pollution, eutrophication, soil acidity, and ecological imbalance (Vimpany and Kelly, 2004). Continuous application of synthetic insecticides also caused a resurgence of pests (Wu et al. 2001; Dutcher 2007; Matsumura and Morimura 2010) due to resistance build-up against insecticides and reduction in natural enemy populations, disrupting the natural balance of insect pests and their natural enemies (Hardin et al. 1995). Furthermore, applying a large amount of chemical fertilizer and pesticide in the agricultural soil reportedly resulted in increased heavy metals like Cadmium (Cd), Lead (Pb), and Arsenic (As) that pose negative effects on the soil fauna (Atafar et al. 2010). Liu et al. (2011) reported a significant reduction in microbial diversity and population in paddy soil due to the intensive application of inorganic inputs.

Organic farming is one of the possible solutions that could address the problems mentioned above while also addressing food and resource sustainability, health, and

environmental issues. It reduces the use of agrochemicals, thereby enhancing productivity without destroying the ecosystem balance and harming farmers, consumers, and the environment, hence observed in this study.

MATERIALS AND METHODS

This study focused only on evaluating the effects of different production systems on pests, natural enemies, and soil microorganisms in the lowland rice field. A total 782 m^2 lowland experimental area at the Department of Agronomy devoted to various production systems for four consecutive croppings with similar treatment applications was used. In addition, a Randomized Complete Block Design (RCBD) with three treatments and four replications separated by a 2 m alleyway used by the previous croppings was retained. Each treatment plot measured 5 m x 6 m with 750 hills of lowland rice spaced at 20 cm x 20 cm.

The following treatments were as follows: (i) T1= Best bet organic production system (green manuring + green leaf manuring + vermicast + vermitea + fermented plant juice (FPJ) + fermented fruit juice + organic insecticide (rumphii "*panyawan*" based extract). (ii) T2= Organic farmers' practice in Leyte (vermicast + FPJ + vermitea + fermented fruit juice + organic insecticides (rumphii "panyawan" based extract). (iii) T3= Conventional farmers' practice in Leyte (urea + complete + Karate a.i. lambda-cyhalothrin + Lannate a.i. methomyl)

Field management and treatment application

A month after harvesting the previous cropping, mungbean (*Vigna radiata* L.) seeds were broadcasted at 30 kg/ha to the best bet organic production system (T1) plots without tilling the soil. When the mungbean reaches the flowering stage, it is plowed and mixed in the soil combined with the kakawate [*Gliricidia sepium* (Jacq.) Kunth] leaves at a rate of 2 kg m^{-2} and allowed to decompose for three weeks before transplanting. Plowing was done twice weekly without disturbing the previous croppings' layout. In addition, dikes and canals around the experimental plot were cleaned, fixed, and repaired.

Three sets of PSB Rc 18 seeds were soaked and incubated separately, and the seeds for T1 were coated with microbial slurry (20% solution of MykoPlus). Pre-germinated seeds for T1 and T2 were sown evenly in a prepared wet bed applied with vermicompost at a rate of 0.5 kg m^{-2}, while T3 seeds were sown in a seedbed applied with complete fertilizer at a rate of 30 g m^{-2}. After sowing, these seedlings were reared and transplanted 21 days after sowing to their respective treatment plots. Seedlings were transplanted at a 20 x 20 cm planting distance. Seedlings planted in T_1 were dipped in the microbial slurry before planting. Replanting was done on the missing hills five days after transplanting (DAT) to maintain the plant population.

FPJ, fruit juice, and vermitea were applied as foliar sprays for T1 and T2. For T1, vermitea and 10% solution of FPJ were sprayed alternately at weekly intervals two weeks after transplanting up to the flowering stage. T2 was applied with a mixture of FPJ and vermitea spray at weekly intervals starting two weeks after transplanting up to the flowering stage at a rate of 30 mL of each foliar supplement per liter of water. At the panicle initiation stage, fermented fruit juice was sprayed at weekly intervals for T1 and T2 at 30 mL per liter of water up to two weeks before the harvesting date. Spraying of foliar fertilizers was done at 4 p.m. when sunlight was not so intense. For T3, synthetic fertilizer at a rate of 109.04 -17.5-17.5 kg N, P_2O_5, K_2O ha^{-1}.

Weeds were controlled manually throughout the experiment, and proper water management was employed. For the pests and diseases, T1 and T2 were sprayed with organic-based insecticides (*Tinospora rumphii*-based extract), while T3 was applied with chemical insecticide lambda-cyhalothrin during the vegetative and methomyl insecticide during the heading stage. Moreover, to prevent contamination of spray mists of chemical pesticides to organic treatments, a plastic enclosure was provided during spraying around the conventional treatment plots.

Statistical analysis

Analysis of variance on data gathered was done using the Statistical Analysis Software (SAS) Version 9.2 developed by SAS Institute. In addition, a comparison of means was made using Tukey's Honestly Significant Difference (HSD) test.

Data gathered

Disease incidence of major rice diseases

Disease incidence was determined at 30, 60, and 90 days after transplanting by counting the number of infected hills within the harvestable area. That was calculated using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{no. of infected hills}}{\text{total no of hills}} \times 100$$

Disease severity of the observed diseases

Rice blast. The severity of blast infection was determined by visual observation of the ten sample plants randomly selected within the harvestable area in each treatment plot using the following rating scale (IRRI 1996).

Bacterial leaf blight. This blight was obtained by measuring the length of the lesion from the 3rd fully expanded leaf of the infected plants.

Table 1. Rice blast scale rating by IRRI (1996)

Rating	Description
0	no infection
1	less than 1% area affected
3	1-5% area affected
5	6-15% area affected
7	26-50% area affected
9	51-100% area affected

The population of insect pests and beneficial insects

This population was determined by sweeping each treatment plot using a swept net. Swept insects were identified and counted with the aid of a hand lens. Sampling was done at 14, 44, and 74 DAT.

Insect damage

This damage was assessed by counting the number of folded/rolled leaves, dead hearts, whiteheads, and other insect damage observed within the harvestable area of each treatment plot throughout the production period.

Grain yield

After cleaning, this yield was determined by weighing the grains from the harvestable area (12 m2) of each treatment plot. In addition, the number of hills harvested in each plot was counted. Moisture content (MC) was determined before weighing using a grain moisture tester. Grain yield was adjusted to 14% MC using the formula:

$$\text{Adjusted Grain Yield at 14\% MC} = \frac{100\% - \text{MC (\%) of grains at harvest}}{100\% - \text{Desired MC (14\%)}} \times \text{Grain yield at harvest (kg)}$$

The weight per plot will be converted to tons per hectare using the formula:

$$\text{Grain yield (t ha}^{-1}) = \frac{\text{Plot yield (kg)}}{\text{Harvestable area (20 m}^2)} \times \frac{10{,}000 \text{ m}^2 \text{ ha}^{-1}}{1{,}000 \text{ kg t}^{-1}}$$

Soil microbial analysis

Soil samples from each treatment plot at a depth of 20 cm were collected using a soil auger. Freshly collected samples were submitted to Plant Disease Diagnostic Laboratory, Visayas State University, Visca, Baybay, Leyte, for microbial identification and colony count at mL^{-1}.

RESULTS AND DISCUSSION

Incidence and severity of rice diseases

The incidence and severity of the major rice diseases throughout the production period are presented in Table 2. Variance analysis showed that tungro disease occurrence was significantly higher in T2, while T1 and T3 are comparable. The higher incidence of tungro in T2 could be due to the lower amount of nutrients applied compared to the other treatments involved in the study. According to Rillon et al. (1998), rice plants treated with additional N fertilizer during the production period showed significantly lower symptoms. They concluded that the application of N enabled the plants to recover from RTSV and RTBV disease, thereby reducing the disease infection.

Lower incidence and severity of bacterial blight and rice blast diseases were observed in T1 and T2 compared to T3. These results could be due to the higher nitrogen applied in the conventional production system, which favored the disease infection spread. Long et al. (2000) reported that applying nitrogen at a higher rate significantly increases blast incidence and total lesion regardless of cultivar. Kurschner (1992) also mentioned that increasing Nitrogen application increases leaf blast due to increases in tissue susceptibility and canopy density. Moreover, Chaudhary et al. (2009) reported that nitrogen application also affects bacterial leaf blight incidence and severity. They found that different N doses caused 6.67 to 55.11% bacterial leaf blight incidence under research field conditions and 7.12 to 62.00% under farmers' field conditions.

The population of insect pests and beneficial insects

The population of five dominant insects throughout the production period: black bug, brown planthopper (BPH), green leafhopper (GLH), grasshopper, and rice bug in the field is presented in Figure 1. The mean number of BPH and GLH showed the lowest in plants under T2 at 14, and 44 DAT, compared to T1.T3 had the highest, which eventually reduced to 74 DAT regardless of treatments. As expected, the mean number of rice bugs greatly increased at 74 DAT when rice had already reached the milking stage. However, a higher number of rice bugs were observed at T_3 while the least in T_1. On the other hand, only fewer grasshoppers and black bugs were observed throughout the production period.

The more GLH and BPH in T_3 is likely due to the higher fertilization. Higher N's application increases BPH's feeding and oviposition (Wang and Wu 1991) and GLH (Karnataka 2011). The increase in the amino acid content of rice sap and more succulent plants due to high nitrogen fertilization could also improve the nutritional conditions for sap-sucking insects, thus increasing their population (Balasubramanian et al. 1983). On the other hand, the reduction of BPH and GLH in T_2 could also be related to the higher population of mirid bugs, as indicated in Figure 2. Manti (1990) reported that a mirid bug is a predatory insect of hoppers' egg that consumes up to 61.23-143.68 eggs in the total lifetime of a single matured mirid bug.

Table 2. Incidence and severity of rice diseases of lowland rice PSB Rc18 as affected by different production systems

Treatments	Tungro incidence	Rice blast		Bacterial blight	
		Incidence	Severity	Incidence	Severity
T1	4.17[b]	2.58[b]	0.27[b]	2.50[b]	18.67[ab]
T2	7.83[a]	2.42[b]	0.15[b]	3.67[b]	11.60[b]
T3	5.42[b]	9.17[a]	1.08[a]	18.25[a]	26.17[a]
Mean	5.80	4.72	0.50	8.14	18.81
CV %	11.61	27.85	22.85	21.25	21.50

Note: Means within each column followed by a common letter and those without designation are not significantly different based on HSD and ANOVA, respectively. Legend: T1: Best bet organic production system, T2: Organic farmers' practice in Leyte, T3: Conventional farmers' practice in Leyte

Moreover, the population of five dominant beneficial insects, namely: damselfly, long-horned grasshopper, lynx spider, mirid bug, and wasp at 14, 44, and 74 DAT, is presented in Figure 2. Results showed that more long-horned grasshoppers and damselfly were noted in T3 at 14 DAT of rice, which subsequently reduced as the crop matured at the succeeding sampling periods. On the other hand, the mirid bug was higher in T2 at 14 and 44 DAT, eventually reducing at 74 DAT.

The Lynx spider population was also higher in both organic production systems (T1 and T2) than in T3 in all sampling periods. On the other hand, higher wasps were observed in T2 at 44 DAT compared to the other production systems. However, no wasp was observed at 14 DAT and fewer at 74 DAT. The reduction of beneficial insects at a later stage (74 DAT) was due to the spraying of organic insecticides (rumphii "panyawan" based extract) in T1 and T2 and chemical insecticide in T3, which either deter or kill the beneficial insects.

The total population of insect pests and beneficial insects at 14, 44, and 74 DAT is presented in Figure 3. The graph shows that the mean number of beneficial insects was higher at 14 and 44 DAT and eventually reduced at 74 DAT. However, the mean number of insect pests increased at 74 DAT while the number of beneficial insects reduced.

The fewer insect pests at an earlier sampling period were possibly due to the reasonable number of beneficial insects and lesser infestation during earlier days of crop establishment. On the other hand, the reduction of beneficial insects in the later sampling period was perhaps due to the spraying of organic insecticide in organic treatments T1 and T2 and chemical insecticide in T3. Therefore, the later increase in insect pests population was mainly attributed to the increased number of rice bugs during the milking stage and the reduction of beneficial insects due to spraying.

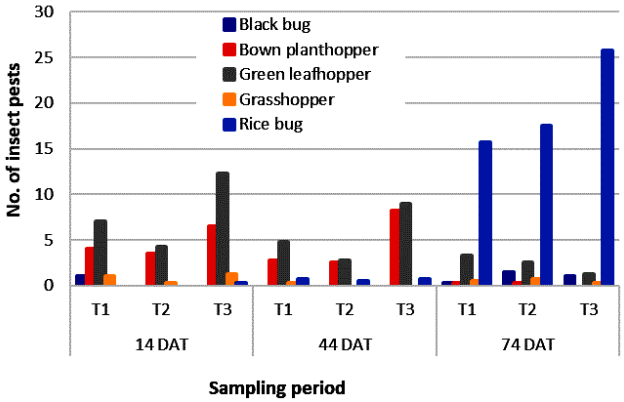

Figure 1. Population assessment of insect pests at 14, 44, and 47 DAT as affected by different production systems

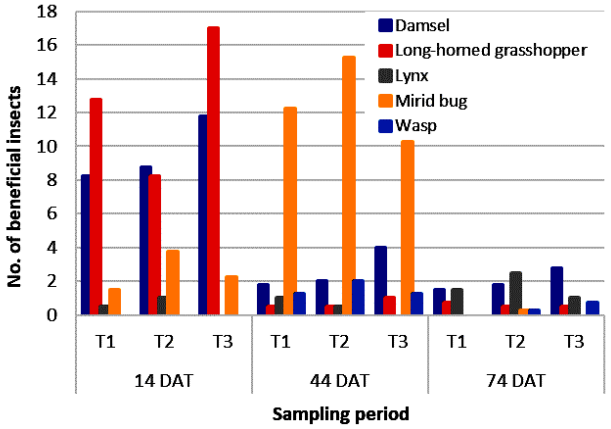

Figure 2. Population assessment of beneficial insects at 14, 44, and 74 DAT as affected by different production systems

Yield and insect damage

Table 3 shows rice yield and insect damage as affected by the different organic production systems throughout the production period. Analysis of variance revealed that there was no significant difference in the number of dead hearts and whiteheads. However, a higher number of folded leaves were observed in T3, which was 93.30% higher than in T2, followed by T1 with 37.50 folded leaves. This result may be attributed to the higher N concentration in conventional farmers' practices in Leyte, which conforms to the findings of Singh and Shahi (1984). They noted an increasing damage rate at increasing N application. For example, at 30 kg nitrogen/ha, the leaf folder damage rate reached 11.03%, while at 60 and 150 kg N/ha, leaf damage increased to 15.33% and 15.06-16%, respectively.

A significantly higher percentage of unfilled spikelets was observed in T1 and T3, while the lowest was in T2. On the other hand, no significant difference was observed in grain yield, indicating a comparable grain yield of rice between organic and inorganic production systems. The higher number of unfilled spikelets is mainly due to the higher number of black bugs and rice bugs in T1 and T3.

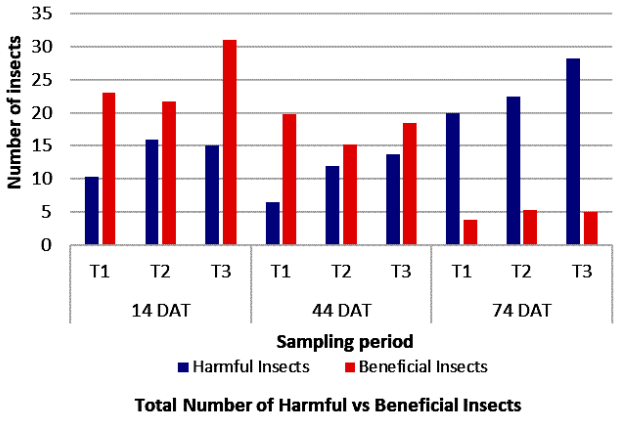

Figure 3. Population assessment of insect pests versus beneficial insects at 14, 44, and 47 DAT as affected by different production systems

Soil microbial analysis

Table 4 presents the microbial counts (cfu mL^{-1}), while Figures 4 and 5 show the bacterial cells and fungi associated with the sample. Initial and final microbial analysis indicated bacterial and fungal microorganisms were associated with the soil samples. Regardless of the treatment, bacterial and fungal colony count mL^{-1} increased at the final analysis relative to the initial count. However, colony-forming unit mL^{-1} of fungi in T1 and T2 showed a reasonably higher increase in count than in T3. The microorganism species associated with T1 were *Bacillus, Chromobacterium, Coccobacillus,* and *Aspergillus*. In T2, *Chromobacterium, Bacillus, Coccobacillus, Aspergillus,* and *Trichoderma* were observed, while T3 observed *Chromobacterium, Bacillus, Coccobacillus, Aspergillus*, and *Fusarium.* These are saprophytic soil-inhabiting microorganisms which are the major decomposers of organic matter (Gomes et al. 2014; Sudrajat et al. 2019). The higher increase of microorganisms in both organic production systems compared to conventional farmers' practices in Leyte could be attributed to the application of organic fertilizer. Bot and Benites (2005) reported that most fungi, bacteria, and actinomycetes rely on organic materials for their carbon and energy needs.

Table 3. Insect damage of lowland rice PSB Rc18 as affected by different production systems

Treatments	Insect damage		Percentage unfilled spikelet panicle^{-1}	Grain yield (t ha^{-1})
	Folded leaves	Dead hearts and whiteheads		
T1	37.50^{b}	44.00	45.04^{a}	3.03
T2	4.50^{c}	69.25	30.43^{b}	3.26
T3	67.25^{a}	42.75	50.15^{a}	3.08
Mean	46.33	52.01	41.87	3.12
CV %	29.75	28.19	13.45	12.75

Note: Means within each column followed by a common letter and those without designation are not significantly different from each other based on HSD and ANOVA, respectively

Table 4. Microbial counts (cfu mL^{-1}) of microorganisms associated with soil samples from different production systems using potato dextrose agar (PDA) and nutrient agar (NA)

Treatment	Microorganism (cfu/mL)			
	Bacteria		Molds/fungi	
	Initial	Final	Initial	Final
T1	1.17x 105	2.33 x 105	3.00 x 102	4.03 x 103
T2	2.60 x 105	4.63 x 105	1.33 x 102	3.00 x 103
T3	2.47 x 105	4.66 x 105	2.00 x 102	1.47 x 103

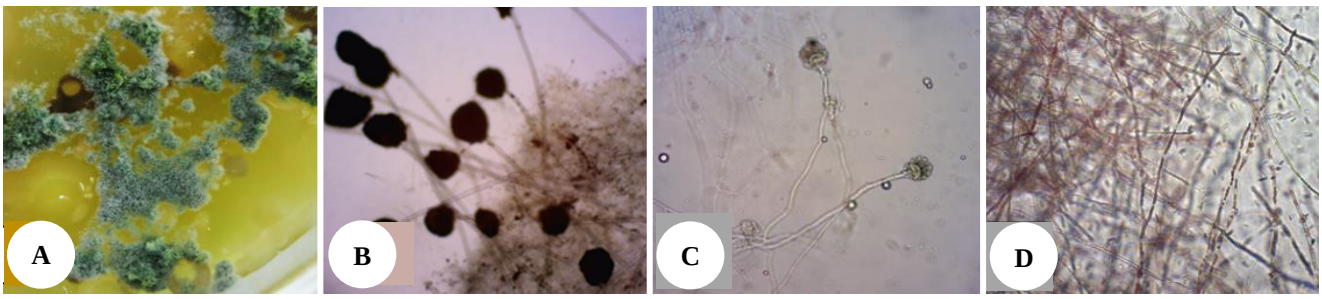

Figure 4. Molds/fungal isolates found associated with soil samples. A. *Trichoderma sp*.; B. *Aspergillus niger*; C. *Aspergillus* sp.; D. *Fusarium* sp.

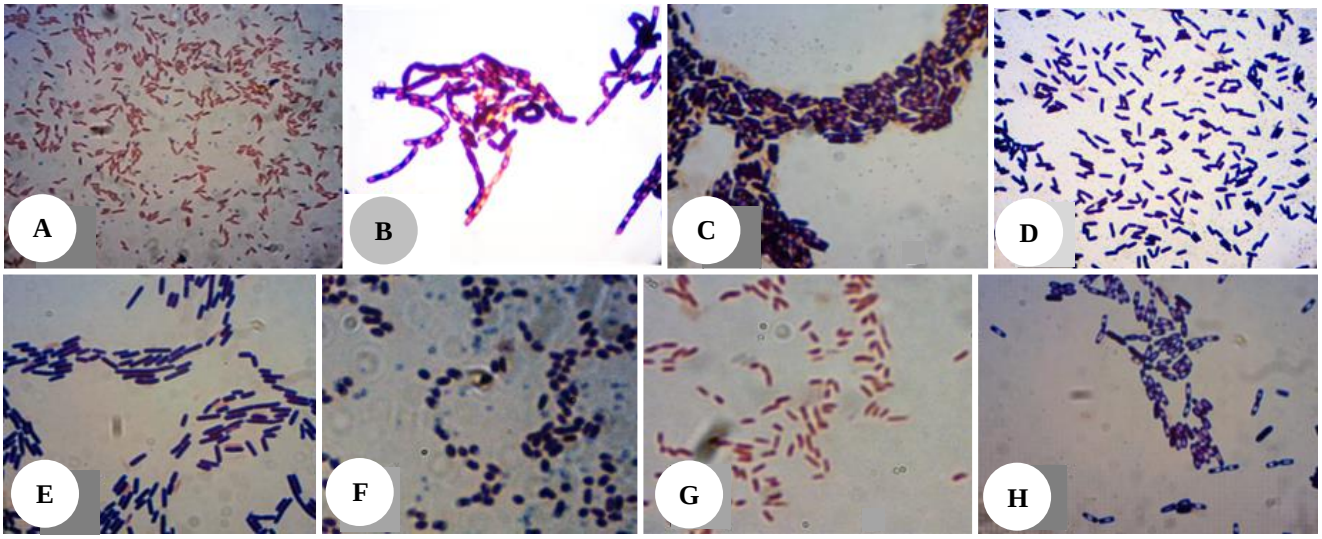

Figure 5. Cells of the bacteria isolate; A. *Chromobacterium* sp.; B. *Bacillus* sp.; C. Flat, a slimy, dirty white colony with irregular margin and Gram-positive rod-shaped endospore-forming *Bacillus* sp.; D. Semi-dry flat, dirty white colony with highly lobate margin, Gram-positive, short rod-shaped, *Bacillus* sp.; E. Dirty white, filamentous and flat colony, Gram-positive rod-shaped *Bacillus* sp.; F. *Cocobacillus* sp.; G. Slimy dirty white lobate colony, Gram-negative rod-shaped *Bacillus* sp.; H. Dirty white, flat, irregular colony, Gram-positive rod-shaped endospore-forming bacterium *Bacillus* sp.

In conclusion, lower incidence and severity of rice blast and bacterial blight but higher tungro virus incidence was observed in rice grown in organic farmer's practice in Leyte compared to the conventional practice. Conventional farmers' practices in Leyte had a slightly higher number of harmful insects such as brown planthopper, green leafhopper, and rice bug. In contrast, organic farming practices in Leyte had the highest number of beneficial insects, like the mirid bug as a predatory insect. The number of folded leaves observed was lowest in organic farmers' practice in Leyte while highest in conventional farmers' practice. The different production systems did not significantly affect the number of dead hearts, whiteheads, and grain yield, but lower unfilled grains were observed in organic farmers' practices in Leyte. The population and diversity of microorganisms are higher in organic farmers' practices in Leyte.

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Construction of an ultra-high-density consensus genetic map and analysis of recombination rate variation in *Sorghum bicolor*

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Abstract. *Satrio RD, Nikmah IA, Fendiyanto MH, Pratami MP, Awwanah M, Sari NIP, Farah N, Nurhadiyanta. 2022. Construction of an ultra-high-density consensus genetic map and analysis of recombination rate variation in* Sorghum bicolor. *Asian J Agric 6: 47-54.* Sorghum is one of the most widely grown cereal crops on a global scale. A consensus map is a method for combining genetic information from multiple populations, and it is an effective way to increase genome coverage and marker density. This study constructed a consensus map by combining publicly available marker data from four mapping populations. A total of 3,449 non-redundant polymorphic markers at the nucleotide level were used to construct a single consensus map on ten sorghum chromosomes. This study generated an ultra-high-density sorghum consensus map consisting of many markers spanning 1,571.68 cM and averaging one marker per 0.46 cM. Due to the markers' high density, only 0.06% of the markers had an interval greater than 5 cM. The local recombination rates were estimated using a set of all markers' genetic and physical positions along each of the ten chromosomes. The analysis of the recombination rate on ten sorghum chromosomes revealed that it decreased as the centromere position was getting closer. The consensus map generated in this study can be used to integrate information related to sorghum genetic resources and QTLs into the genome sequence, thereby accelerating the discovery of novel potential genes in sorghum.

Keywords: Genetic map, linkage, recombination rate, single nucleotide polymorphism, *Sorghum bicolor*

INTRODUCTION

Sorghum is a major cereal crop on a global scale, often placing fifth in yearly volume (FAOSTAT 2021). Due to crop resistance to a broad range of biotic and abiotic stressors, sorghum is widely planted in marginal cropping zones and water-scarce conditions in developed and developing countries (Leff et al. 2004). Sorghum is a staple food and a source of fodder in underdeveloped countries for the impoverished. In industrialized nations, it is mostly utilized as animal feed. Sorghum cultivars adapted in tropical regions have generated significant relations as a potential cellulosic biofuel-producing plant (Vermerris 2011). Sorghum genetic improvement projects worldwide are attempting to increase varieties quality, disease resistance, drought tolerance, and agronomic features (Bernardino et al. 2019, 2021). Molecular-assisted breeding techniques are broadly used to construct linkage maps and discover chromosomal regions associated with essential sorghum traits, like stay-green, disease resistance, abiotic stress tolerance, high yield productivity, and photoperiod insensitivity (Harris et al. 2007; Morris et al. 2013; Girma et al. 2019).

High-density genetic or linkage maps are necessary to investigate the inheritance of qualitative and quantitative traits, design markers for molecular breeding, perform map-based gene cloning, and conduct comparative genomic investigations. Molecular breeding is more effective when a densely-marked molecular map (Hufnagel et al. 2018). In addition, the consensus genetic map increases the diversity and quality of markers and the frequency of polymorphic markers at important chromosomal intervals. In the early 1990s, the sorghum genome was genetically mapped using DNA markers, and multiple genetic linkage maps were published during the previous decade (Mace et al. 2009, 2019). Early maps were constructed using Restriction Fragment Length Polymorphism (RFLP) markers, but more recent maps have incorporated Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Diversity Array Technology (DArT), and, more recently, microarray and sequencing-based Single Nucleotide Polymorphism (SNP) markers (Pennisi 2017; Miftahudin et al. 2021b).

The development of innovative marker technologies enables rapid and sequence-independent whole-genome analyses of any plant species. Due to the massively parallel and automated nature of the high-throughput sequencing (HTS) technique, the cost per data point is lowered massively compared to earlier technologies (Elshire et al. 2011). Moreover, the HTS-based marker permits direct incorporation into the reference sequence for the sorghum genome (Bouchet et al. 2012). Therefore, integration of the constantly growing number of genetic linkage data generated by various novel marker technologies is

essential. Aside from that, the vast majority of published sorghum genetic linkage maps were constructed using wider crossovers than the number of crossings generated in sorghum breeding projects (Mester et al. 2015; Bouchet et al. 2017). However, genetic linkage maps derived from large crossings are typically insufficient for molecular breeding methods because they do not adequately represent the gene pool's genome architecture and function. In addition to providing an invaluable reference resource, constructing a consensus map by combining data from several mapping populations with various genetic constitutions also provides for mapping a greater number of loci than is possible in most single crosses. Thus, the number of potentially usable markers is increased across various genetic backgrounds and genome coverage levels while simultaneously offering chances to confirm marker order (Mace et al. 2009; Qu et al. 2021).

On a genetic map, the distance between markers is proportional to the frequency of recombination. Meiotic homologous recombination is crucial in plant breeding because it generates novel combinations of preexisting genetic diversity. Over the past decade, our knowledge of meiotic recombination and genomic diversity in plants has evolved dramatically (Lambing et al. 2017). DNA sequencing technology has allowed the discovery of high-resolution genetic information in plant genomes, boosting plant breeding methods via high-throughput genotyping, linkage analysis, and association mapping. The genetic distance was divided by the physical distance to determine the recombination rate using linkage disequilibrium mapping and molecular marker-based linkage mapping (Apuli et al. 2020). Understanding the genome distribution of recombination rates helps forecast the population's potential response to environmental change in quantity and breeding strategy (Shen et al. 2017).

The research aimed to integrate the linkage maps obtained from four distinct maps in sorghum and construct them into a single consensus genetic map. Additionally, the rate of local recombination of the chromosomes was evaluated. The consensus genetic map constructed in this study can be linked to a well-annotated reference physical map that may accelerate gene discovery in sorghum.

MATERIALS AND METHODS

Data collection

A total of four *Sorghum bicolor* (L.) Moench mapping populations were utilized to incorporate approximately 5000 individual loci (Table 1), predominantly SNP markers, into a single consensus map. The BTx623 was chosen as one of the population parents for the sorghum genome sequencing research by Kong et al. (2018). The usage of BTx623 streamlines the process of integrating the consensus genetic map generated in this work with the publicly accessible physical map sequence. Three additional mapping populations with a varied parental line were employed in this analysis, including the F_2 population used by Ji et al. (2017), the RIL population used by Lopez et al. (2017), and Phuong et al. (2019).

Consensus map integration

We constructed a consensus map for sorghum using four different genetic maps based on SNP, SLAF, DArT, and SSR markers, which combined to form a single map. Before integrating SLAF, DArT, and SSR markers into a single consensus map, they were converted to SNP markers. The nucleotide sequences of all primers from those markers were compared using an automated batch BLASTN search (Altschul et al. 1990), with E < 1e-10 against the BTx623 sequence as the reference genome (McCormick et al. 2018). The best hit for each marker was chosen to derive the map position by integrating the positions of adjacent markers. The average of each marker's start and end coordinates were used to determine its physical location throughout the genome. The actual marker locations relative to the reference genome were identified using a custom Perl script. Additionally, numerous markers were removed from the downstream analysis since their physical position could not be determined. Consensus maps for each sorghum chromosome were generated using the LPmerge package (Endelman and Plomion 2014) of R software. This tool extensively uses linear programming to reduce the mean absolute error associated with integrating many genetic or linkage maps.

Estimation of recombination rates

After finding the physical placements of the markers and integrating them genetically, the rates of local recombination along each of the ten chromosomes were assessed using MareyMap (Rezvoy et al. 2007). To demonstrate the relationship between genetic and physical positions, a scatter plot was used to compare the genetic (cM) and physical (Mb) locations of the markers (Shen et al. 2017). The Loess technique was used to create and show the recombination map for each marker through MareyMap. The centromere positions for each chromosome were determined using Evans et al. (2013) physical map. The correlation between the position of genetic markers and their physical map was evaluated using Spearman correlation (Sedgwick 2014).

Table 1. A summary of the mapping data used to construct the consensus map for sorghum

Study	Type of Population	Number of individuals	Parental of population	Type of marker	Number of makers	Length of a genetic map (cM)
Kong et al. (2018)	RIL	399	BTx623 × IS3620C	SNP	381	1408.8
Ji et al. (2017)	F2	130	Keter × J204	SLAF	2246	2158.1
Phuong et al. (2019)	RIL	140	HYP × DYP	DArT, SSR	184, 9	1212.0
Lopez et al. (2017)	RIL	135	Early Hegari-Sart × Bk7	SNP	2833	1559.9

RESULTS AND DISCUSSION

Consensus genetic map of *Sorghum bicolor*

Multiple mapping investigations of the sorghum genome using DNA markers have been conducted over the last decade, first using RFLP markers and, more recently, using AFLPs, SSRs, DArT, and, most recently, SNP markers (Nadeem et al. 2018). Integrating SNPs' constantly expanding set of linkage data with the numerous genetic linkage maps is important for sorghum gene identification. The objective of this article was to examine the collinearity of four independent sorghum component maps and integrate them into a single reference resource using many markers. The maps with four components were generated using the LPmerge package of R software. The lengths of the published individual maps varied from 1212 to 2158 cM (Table 1). A weighted technique based on population size implemented in LPmerge was used to construct the framework consensus map. The Sorghum consensus map comprised 3449 markers covering 1571.68 cM and an average of one marker per 0.46 cM (Figure 2).

The markers and their genetic and physical position on ten sorghum chromosomes were deposited in the Zenodo (DOI: 10.5281/zenodo.3474022). A total of 2204 markers were omitted from the newly developed consensus map or were determined redundant with those generated across studies in this meta-analysis. These markers were removed and not included in developing the consensus map process. The consensus map contains an average of 345 markers per chromosome. Non-random patterns were discovered in the distribution of DNA markers, with some locations visibly rich with markers and others being sparse (Figure 1). The typical distance between markers was quite short; most chromosomes were separated by less than 5 cM (Figure 2). On chromosome 7, however, there was still a 9.52 cM interval. The low recombination rate may account for the region's difficulty in mapping. Our result improves the consensus genetic linkage map previously constructed using combined RFLP, AFLP, SSR, and DArT markers (Mace et al. 2009). The number of markers increased from 2029 to 3449, and the mean markers density was narrowed from 0.79 to 0.46 markers per cM. The genetical chromosome size was relatively similar, i.e., 1603.5 and 1571.7 cM in previous and current studies.

The sorghum genome has 818 Gb of DNA and is made up of 10 chromosomes (Paterson et al. 2009). Sorghum chromosomes have vast pericentromeric regions encompassing around 50% of the genome and were characterized by low gene density and low recombination rates. Euchromatic DNA has a higher number of genes since it covers the outermost section of each chromosome arm. The current reference Tx623 genome assembly spans 720 Mb of DNA, consisting of 10 sorghum chromosomes (683.65 Mb) and several small large-contigs that were not integrated into the reference genome sequence (Cooper et al. 2019; Ruperao et al. 2021). The final consensus map enabled us to map more markers than any individual, get more comprehensive genome coverage, and complete several gaps in individual maps.

The final consensus map allowed us to map more markers than any individual map, acquire a more comprehensive coverage of the sorghum genome and complete multiple gaps in previously published maps (Ji et al. 2017; Lopez et al. 2017; Kong et al. 2018; Phuong et al. 2019). Apart from the fact that the sequence of markers was consistent across individual component maps, excellent agreement in the total distances between common marker pairs was discovered throughout the component maps utilized in this investigation using a different ratio approach (Zhang et al. 2018; Hu et al. 2021). The generated consensus genetic map may be used as a reference for genomic investigations in individuals with various genetic origins and a framework for genetic data transfer across various marker technologies and for combining SNP markers with other genomic resources. The SNP markers are a low-cost, high-throughput marker technology that is especially beneficial in genetic mapping, through QTL mapping or association study, and molecular breeding efforts for crops such as sorghum.

The ultra-high-density consensus genetic map constructed in this study could be used to facilitate QTL mapping to discover novel genes controlling valuable traits. The traits can be observed in agronomy (Satrio et al. 2021), morphology (Fendiyanto et al. 2019a; Miftahudin et al. 2021a), physiology (Fendiyanto et al. 2019b), metabolites (Fendiyanto et al. 2020, 2021b), or transcriptional level (Satrio et al. 2019; Fendiyanto et al. 2021a; Ratnadewi et al. 2021). In addition, published QTL maps from any previous study could be integrated with the consensus genetic linkage map constructed in this study as the meta-QTL analysis. QTLs detected from meta-QTL analysis generally have higher robustness than individual QTL studies, such as in the cases of wheat (Hu et al. 2021), rice (Khowaja and Price 2008; Trijatmiko et al. 2014), potato (Danan et al. 2011), and wheat (Qu et al. 2021).

Recombination rate estimates in the sorghum genome

The collinearity of the consensus genetic map and the physical map depicted in Figure 2 were studied on all sorghum chromosomes. The findings suggested that these markers were quite useful for building a genomic map. The bulk of chromosomal markers overlapped significantly between the genetic and physical maps. The Spearman correlation coefficient was typically consistent with the degree of genetic collinearity between each genetic and physical location. On all chromosomes except for chromosomes 7 and 10, the Spearman correlation value was more than 0.95. A high Spearman correlation coefficient suggested a significant association (Shen et al. 2017).

The topography of recombination rate variation throughout the genome is shown in Figure 2, which calculates the rate of recombination per physical distance (Mb) along each of the ten sorghum chromosomes. The consensus linkage map created in this research was used to calculate recombination estimates. The average genome-wide recombination rate did not follow a random distribution, and recombination occurred at a greater rate in distal chromosomal areas than in proximal ones. One

broad-scale and persistent trend within chromosomes was a lower recombination rate at centromeres. While this might be explained by selection against recombination in highly repeated areas, a repetitive sequence is not essential. Moreover, species lacking or with few centromeric repeats also demonstrate decreased centromere recombination (Stapley et al. 2017). Suppression is probably definitely a function of chromatin shape; double-strand DNA breaks are less prevalent in condensed heterochromatin. The chromatin environment may affect the chance that a double-strand break is repaired without a crossover. A double-strand break and subsequent repair by a non-crossover may be widespread in centromeres. That might account for the accumulation of repetitive components and diversity of centromeres despite the apparent paucity of crossings (Talbert and Henikoff 2010).

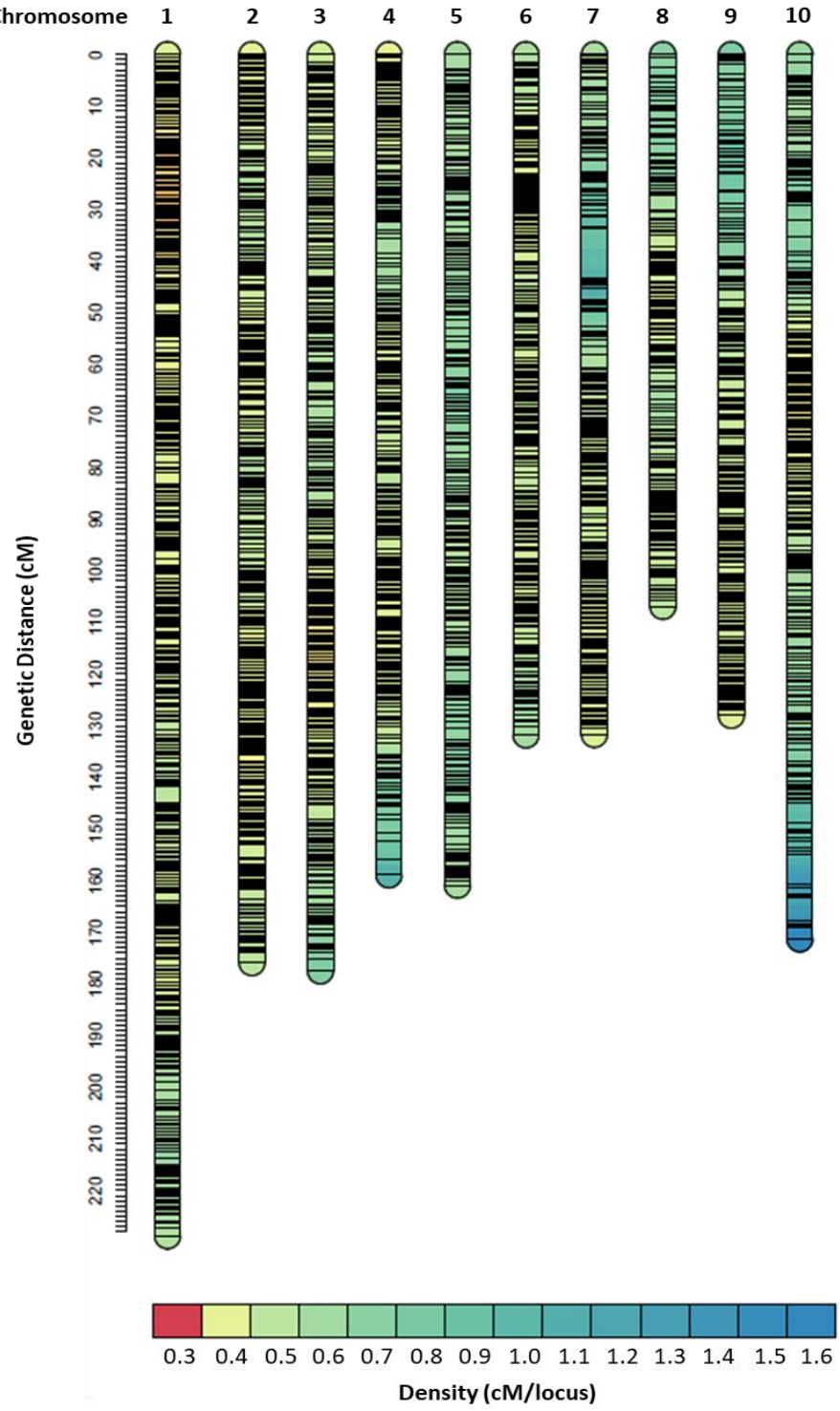

Figure 1. Ultra-high-density consensus genetic map of 10 sorghum chromosomes constructed from a large number of markers

Table 2. Characteristics of the ultra-high-density consensus genetic map constructed from a large number of markers in sorghum

Chr.	Physical chr. size (Mb)	Genetical chr. size (cM)	Number of markers	Average distance between markers (cM)	Maximum distance between markers (cM)	Gap < 5 cM (%)
1	80.88	228.84	591	0.39	3.14	100
2	77.74	175.87	430	0.41	2.54	100
3	74.39	177.46	414	0.43	2.89	100
4	68.66	158.92	350	0.46	3.77	100
5	71.85	160.92	288	0.56	2.65	100
6	61.28	131.81	308	0.43	1.86	100
7	65.51	131.70	266	0.49	9.52	99.62
8	62.69	106.88	222	0.48	3.09	100
9	59.42	128.01	267	0.48	3.11	100
10	61.23	171.27	313	0.55	5.67	99.68
Whole	683.65	1571.68	3449	0.46	9.52	99.94

Note: Gap < 5 cM: the average distance between neighboring markers that is less than 5 cM; Chr. = chromosome

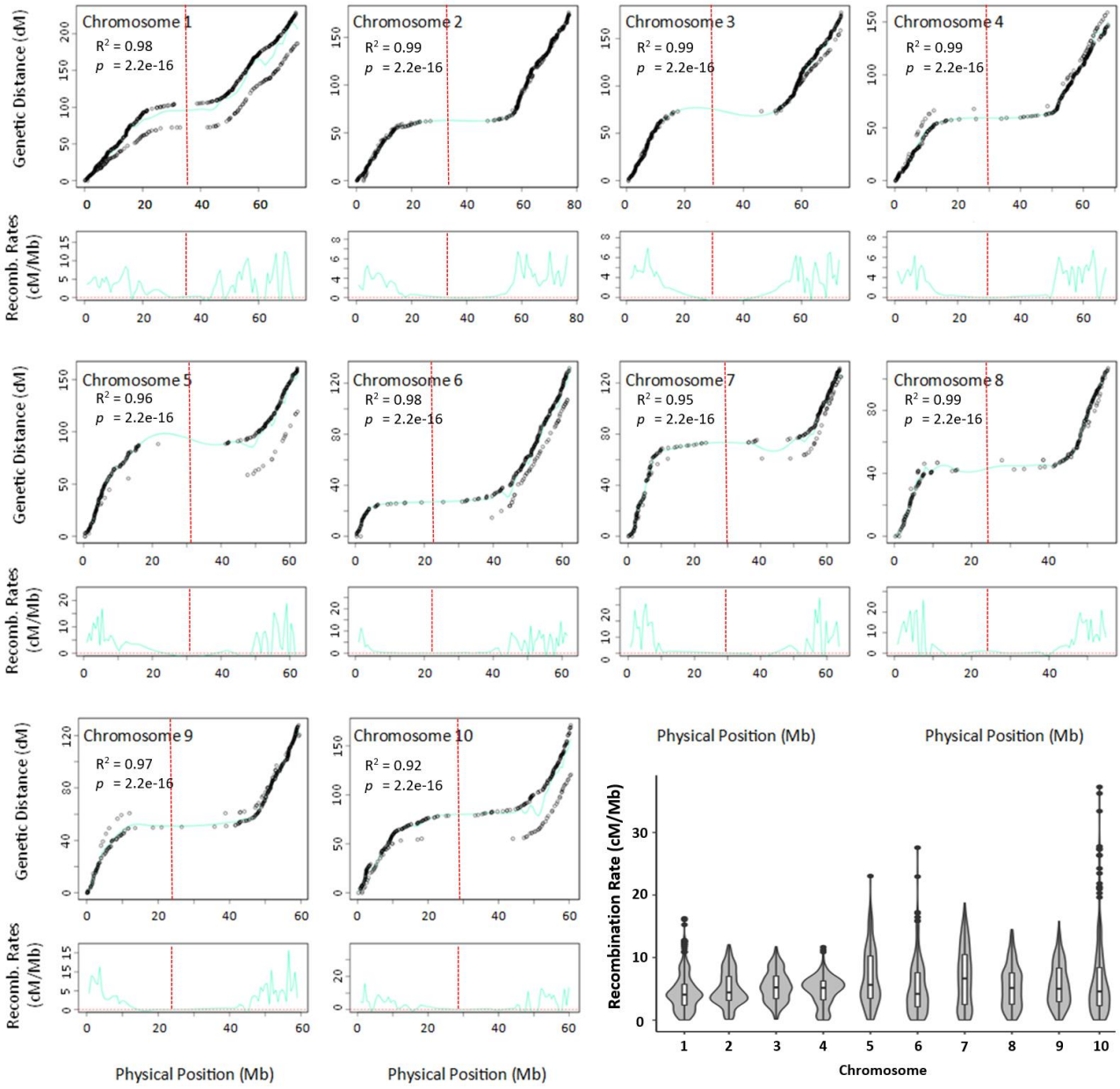

Figure 2. The relationship of genetic and physical maps and their estimated local recombination rates of the sorghum genome. The R^2 value represents the Spearman correlation coefficient between the genetic and physical maps. The light blue curves below the scatter plots represent the estimated local recombination rates. The dashed red line indicates the position of the centromere for each chromosome. The summary of the recombination rates in the whole genome of sorghum was represented as the violin and box plot

The rate of recombination differed across chromosomes (Figure 2). In summary, recombination rates varied on chromosomes 4, 6, and 8 from 0.01 cM/Mb to 37.28 cM/Mb. The calculated median rate of recombination was 4.83 cM/Mb. The estimated mean rate of recombination was 5.44 cM/Mb. Recombination rates computed using the consensus linkage map and polymorphism data revealed a large variance across all chromosomes on Mb scales (Figure 2). Most of our observations fell within the range of 0-37.28 cM/Mb for the consensus genetic map-based estimates, which is consistent with what was observed in other plants, such as *Arabidopsis thaliana (*L.) Heynh. (Giraut et al. 2011), *Populus trichocarpa* Torr. & A.Gray ex. Hook. (Slavov et al. 2012), and *Eucalyptus grandis* W.Hill (Silva-Junior and Grattapaglia 2015). Additionally, recombination of hot and cold spots have been observed in *Zea mays* L. and *Oryza sativa* L. (He and Dooner 2009). However, recombination hotspots or coldspots are typically reasonably modest in size, spanning just a few Kb (Choi and Henderson 2015). Sorghum's average recombination rate is several times that of many other plant and animal species (Tiley and Burleigh 2015), meaning that it possesses one of the greatest recombination rates ever reported in the plant kingdom.

Our high-density sorghum consensus genetic map was a useful resource for *S. bicolor* and comparative genomics investigations within the genus *Sorghum*. Recombination estimates rates generated from a consensus genetic map constructed from various linkage maps were generally consistent across studies. Our results imply that, since recombination estimates were based on population-scale variation, they may be especially helpful for discovering fine-scale recombination variation and identifying hot- or coldspots recombination in the genome. Thus, more study is necessary to ascertain the relative role of positive and negative selection in sculpting sorghum genome-wide diversity, and having access to the tools produced here would aid these investigations.

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