AGROLOGIA, vol. 12, no. 1, pp. 1-8, 2023.
e-ISSN 2580-9636
DOI: http://dx.doi.org/10.30598/ajibt.v11i2

Populasi Azotobacter dan Pseudomonas di rizosfer dan Hasil Caisim setelah Aplikasi Pupuk Kimia dan Pupuk Hayati

Reginawanti Hindersah*, Adita Rizky Syntianis, Ade Setiawan, Rina Devnita, Nursuhud

Fakultas Pertanian Universitas Padjadjaran. Jalan Raya Bandung-Sumedang Km. 21 Jatinangor, Sumedang
**corresponding*: reginawanti@unpad.ac.id

ABSTRAK

Inokulasi mikroba menguntungkan merupakan salah satu cara untuk meningkatkan populasi rhizobakteri yang berperan dalam siklus hara dan menurunkan dosis pupuk kimia. Percobaan pot telah dilakukan untuk mengevaluasi pengaruh pupuk hayati konsorsium yang dikombinasikan dengan berbagai dosis pupuk majemuk NPK terhadap populasi Azotobacter sp. dan pelarut fosfat Pseudomonas sp. di rizosfer dan hasil caisim (Brassica juncea L.). Percobaan disusun dalam Rancangan Acak Lengkap yang terdiri dari sepuluh kombinasi berbagai dosis pupuk hayati dan pupuk anorganik; dan tiga ulangan. Hasil percobaan menunjukkan bahwa inokulasi pupuk hayati dengan maupun tanpa pupuk kimia tidak meningkatkan populasi Azotobacter dan Pseudomonas tetapi berpengaruh terhadap bobot segar tajuk. Tanaman yang diberi ¾ dosis NPK dikombinasikan dengan ½ atau ¾ dosis pupuk hayati; dan kombinasi pupuk NPK dan pupuk hayati dengan dosis anjuran menghasilkan bobot tajuk lebih tinggi daripada kontrol dan perlakuan lainnya. Percobaan ini menunjukkan bahwa pupuk hayati konsorsia bakteri pemfiksasi nitrogen dan pelarut fosfat dapat menurunkan dosis pupuk NPK sampai 75%.

Kata Kunci: bakteri pelarut fosfat, bakteri pemfiksasi Nitrogen, bobot tajuk, pupuk hayati konsorsia

Azotobacter and Pseudomonas Population in Rhizosphere and Yield of Choy Sum following Chemical Fertilizer and Biofertilizer application

ABSTRACT

.Inoculation of beneficial microbes is a way to improve the population of rhizobacteria that has a role in nutrient cycle and hence reduce the level of chemical fertilizer. A pot experiment was performed to evaluate the effect of and consortia biofertilizer combined with various dose of NPK compound fertilizer on the population of nitrogen-fixing Azotobacter sp. and phosphate solubilizing Pseudomonas sp. in rhizosphere and yield of choy sum (*Brassica juncea* L.). The experimental was setup in completely randomized block design consisted of ten combinations of various biofertilizer and inorganic fertilizer dose; and three replications. The results showed that Biofertilizer inoculation with or without chemical fertilizer did not improve the population of Azotobacter and Pseudomonas in the rhizosphere but increase the shoot weight of choy sum. Higher shoot weight was showed by crops with ¾ dose of NPK combined with recommended or reduced dose of fertilizer; and recommended dose of NPK and biofertilizer. This experiment verified that the used of consortia biofertilizer composed of Nitrogen-fixing bacteria and phosphate-solubilizing microbes reduced 50-75% dose of NPK.

Keywords : liquid consortia biofertilizer, nitrogen-fixing bacteria, phosphate solubilizing bacteria, Shoot weight

INTRODUCTION

Inorganic fertilizers play a role in increasing the availability of nitrogen (N) and phosphorus (P) in tropical soils which are generally low in N and P. Currently NPK compound fertilizers are used intensively by farmers because they supply nutrients quickly, and are easy to handling and apply. However, inorganic fertilizers are non-renewable and require a lot of energy in their production process. Reducing the dosage of inorganic fertilizers with biofertilizers has been suggested for replicable and sustainable agriculture. Microbes in biofertilizer formulas are generally N-fixing and phosphate-solubilizing bacteria (NFB). The N fixation is an enzymatic process by which atmospheric N_2 is converted through nitrogenase into available N (ammonium and nitrate) for plants uptake [1]. Many soil phosphate solubilizing microbes (PSM) is proposed to resolve the P deficiency soil [2]; since they are capable of convert insoluble P into soluble P through the production of organic acids [3].

The N-fixing microbes that have been extensively studied and used in biofertilizer formulas are the non-symbiotic NFB Azotobacter and Azospirillum. The fixation capacity of both non-symbiotic bacteria is approximately 25 - 73.8 kg/ha/year and 24.63-134.29 nmol C_2H_4 g/h are reported [4,5]. which has the potential to reduce the use of nitrogen fertilizers. The N fixation process takes place at steady state, so it does not require energy to increase temperature and pressure as in the urea fertilizer industry. The ability of PSM to provide phosphate ions by excreting organic acid is an enzymatic way for reducing the dose of phosphate fertilizers. Moreover, increasing available P in soil could reduce the dose of P fertilizer. The Pseudomonas and Bacillus are among important PSB in soil [3,6,7].

Both NFB an PSM are microbes in the rhizosphere that also synthesize phytohormones and exopolysaccharides. Phytohormones play an important role in regulating cell formation and division, such as auxin, cytokinin and gibberellin are growth hormones produced by certain plant growth promoter bacteria and fungi [8, 9]. Azotobacter bacteria produce phytohormones indole acetic acid and (auxin) zeatin (cytokinins) during in vitro culture [10]. Production of phytohormones auxin by Pseudomonas bacteria has also been confirmed [11]. Exopolysaccharides are produced by rhizobacteria such as Rhizobium, Azotobacter and Pseudomonas which play an important role in plant production in harsh environment [12]. The EPS production by Acinetobacter and Penicillium have also been reported [13,14].

In sustainable agriculture, reducing the chemical fertilizer and balance composition of fertilizer is needed in order to maintain soil health and plant productivity [15]. Moreover, organic matter amendment is oblige to increase the level soil organic-C and to support microbial growth. In general, biofertilizer composed of heterotrophic microbes that depend on organic-C for their metabolis [16]. Organic matter is also prominent in leafy vegetable production; it increases the soil porosity to support hairy roots of vegetable [17].

Almost in all region of Indonesia, leafy vegetable choy sum (*Brassica juncea* L) is consumed in huge quantity. Therefore, used of biofertilizers are important to decrease the use of inorganic fertilizer in choy sum cultivation. The objective of pot experiment was to observe the role of some dose of consortia biofertilizer combined with various dose of NPK fertilizer to improve the population of nitrogen-fixing *Azotobacter* sp. and phosphate solubilizing *Pseudomonas* sp. in rhizosphere; as well as yield of choy sum.

MATERIALS AND METHODS

The experiment has been conducted in the greenhouse belong to Faculty of Agriculture Universitas Padjadjaran in Jatinangor Campus with the altitude of 726 m above sea level. 19-days old choy sum seedlings were grown in 2 kg of potted soil mixed with cow manure equal to 20 kg/ha. The soil was clay Inceptisols (10.26% sand, 26.85% silt and 50.89% clay) collected from natural environment nearest the greenhouse. The soil was neutral (pH 6.8) with 0.75% organik-C, 0.1% total-N, C/N 7.08, 2.27 mg/100 g potential-P_2O_5, 5.56 mg/kg available P_2O_5, and 9.41 mg/100g potential K_2O.

The bacterial count was carried out in the Laboratory of Soil Biology, Faculty of Agriculture. The soil and manure chemical characteristics were analyzed before experiment with the proximate analysis according to Official Methods of Analysis of

AGROLOGIA, vol. 12, no. 1, pp. 1-8, 2023.
e-ISSN 2580-9636
DOI: http://dx.doi.org/10.30598/ajibt.v11i2

the Association of Analytical Communities [16]. The chemical properties of soil and manure determined the fertilizer and biofertilizer treatments in this experiment.

Experimental design

The pot experiment was setup in randomized block design with 10 combination treatments of various dose of NPK and biofertilizer (BF) (Table 1). The treatments were determined based on soil chemical characteristics; which is infertile soil low in N, P and K. The amount of each fertilizer added to individual pot were calculated based on planting distance in field; 20 cm x 20 cm.

Tabel 1. Amount of liquid biofertilizer and compound NPK added to potted soil of each treatment

Code	Fertilizer treatments	Amount of fertilizer per potted soil	
		NPK (g)	BF[3] (mL)
A	Control (without fertilizer)	0	0
B	1 dose of NPK[1]	1.5	0
C	1 dose of biofertilizer (BF)[2]	0	2.5
D	¼ dose of NPK + 1 dose of BF	0.375	2.5
E	½ dose of NPK + 1 dose of BF	0.75	2.5
F	¾ dose of NPK + 1 dose of BF	1.125	2.5
G	1 dose of NPK + 1 dose of BF	1.5	2.5
H	¾ dose of NPK + ¾ dose of BF	1.125	1.875
I	¾ dose of NPK + ½ dose of BF	1.125	1.25
J	¾ dose of NPK + ¼ dose of BF	1.125	0.625

Note: [1]Recommended dose of 300 kg ha^{-1}; [2]Recommended dose of 5L/ha.

Liquid biofertilizer composed of N-fixing bacteria Azotobacter, Azospirillum and Acinetobacter; and phosphate-solubilizing microbes Pseudomonas and Penicillium provided by Laboratory of Soil Biology.

Experimental Establishment

Soil was collected with composite method from the field at a depth of 0-20 cm as much as 7 kg, cleaned from plant residues and debrie, air-dried, and pounded and filtered through a 2-mm sieve. A total of 500 g of soil were put in sealed plastic bag and put at room temperature prior to chemical analysis. Potted soils were prepared by adding 2 kg of soil into 25 cm x 25 cm polybag; then enriched with 60 g of cow manure.

The seedlings of choy sum seed cultivar Shinta were prepared in seedling tray contained of mixed of soil and manure (1:1; v:v) for 19 days. Single seedlings were grown in potted soil with water content of field capacity; all plants were stored in the greenhouse for 25 days. The NPK fertilizer was added twice at 5 and 15 days after planting with split doses; meanwhile the biofertilizer was diluted in water by 1% and pour into the soil surface around the stem at day 7 and day 17 with half dose each.

Parameters and Statistical Analysis

At 25 days after planting, fresh weight of shoot, plant height and soil pH were measured. At the same time, the rhizosphere soil was collected and put in sealed plastic bag and stored at 4^0C before bacterial counting by using serial dilution plate method [18]. The agar media for Azotobacter and Pseudomonas counting was N-free Ashby's mannitol and Pikovskaya respectively. The presence of Azotobacter in plat agar were determined by white, transparent, round and raised colony. The characteristics of Phosphate solubilizing Pseudomonas in Pikovskaya was the halo zone around the white-opaque-raised colony. All data were subjected to analysis of variance with $p \leq 0.05$ followed with Tukey test ($p \leq 0.05$) if the sum square of treatments were significantly affected the parameter

RESULTS AND DISCUSSION

Soil and Manure Chemical Properties

The soil reaction is neutral (6.84) but the soil has very low organic-C (0.75%) and total-N (0.10%) resulted in low C to N ratio (7.08). Furthermore, the content of potential phosphor (P) and potassium (K) in soil were very low (2.27 and 9.41 mg/100 g respectively) and the available P was low (5.46 mg/kg). The cation exchange capacity (22.52 mg/100g) and base saturation (66%) of soil were medium. In overall, the soil was infertile with low organic matter and major essential nutrient N, P and K.

Based on the soil and manure properties, the better NPK for this experiment were that with high content of N since the N soil was low. The low content of organic-C in soil can be compensated by adding organic matter with proper C/N to avoid high release of N from manure. In order to fit the need of nutrient and plant root growth, the NPK 16:11:11 was used at 300 kg/ha as recommended for leavy vegetable. The dose of manure amended before transplanting was 15 kg/ha (60 g per pot). The manure had pH 7.04, water content of 28.53%, organic-C 18.66%, total-N 1.40% and C/N 13.3.

Population of Azotobacter and Pseudomonas

Analysis of variance found that the population of both bacteria have not affected by any fertilizer treatments. Even in soil without any fertilizer, the Azotobacter and Pseudomonas population was similar with treated soil. However, irrespective of Tukey test, Azotobacter population of ¼ dose of NPK + 1 dose of BF and ¾ dose of NPK + 1 dose of BF were increased by 78.2% and 54% compared to control; meanwhile, the high Pseudomonas population was recorded in the rhizosphere of choy sum grown in soil with ¾ dose of NPK + 1 dose of BF; which is 31.6% higher than control (Table 2). Recommended dose of biofertilizer was possibly optmum for Pseudomonas growth, moreover the P content in ¾ dose of NPK fertilizer was not too high to repress the growth of P-solubilizing Pseudomonas.

Plant Growth and Yield

The pest that attacked the leaves was the caterpillar *Plutella xylostella* (Figure 1) that were found on the underside of the leaves and began to attack the plants 6 days after planting. Leaf vegetables from the Brassiceae family are susceptible to *P. xylostella* attack since they are the host plants of this caterpillar [19]. Despite P xylostella attack, the plants were normally growing; at the end of experiment the control plants had lower height compared to plant received fertilizer (Figure 2). This experiment found that the fresh weight of shoot (edible part) as well as plant height was influenced by combination of NPK and biofertilizer (Table 3).

AGROLOGIA, vol. 12, no. 1, pp. 1-8, 2023.
e-ISSN 2580-9636
DOI: http://dx.doi.org/10.30598/ajibt.v11i2

Tabel 2. Effect of NPK fertilizer application combined with biofertilizer concortia on shoot fresh weight and height at 25 days after transplanting

Fertilizer treatments	Azotobacter population (10^4 CFU/g)	Pseudomonas population (10^6 CFU/g)
A = Control (without fertilizer)	9.2 a	15.8 a
B = 1 dose of NPK	8.6 a	14.9 a
C = 1 dose of biofertilizer (BF)	9.8 a	8.3 a
D = ¼ dose of NPK + 1 dose of BF	16.4 a	16.7 a
E = ½ dose of NPK + 1 dose of BF	11.1 a	14.2 a
F = ¾ dose of NPK + 1 dose of BF	14.2 a	20.8 a
G = 1 dose of NPK + 1 dose of BF	8,9 a	6.7 a
H = ¾ dose of NPK + ¾ dose of BF	9.8 a	11.7 a
I = ¾ dose of NPK + ½ dose of BF	11.4 a	10.8 a
J = ¾ dose of NPK + ¼ dose of BF	8.4 a	10.1 a

Note: Values followed by the same letters were not significantly difference based on Tukey test at $p \leq 0.05$.

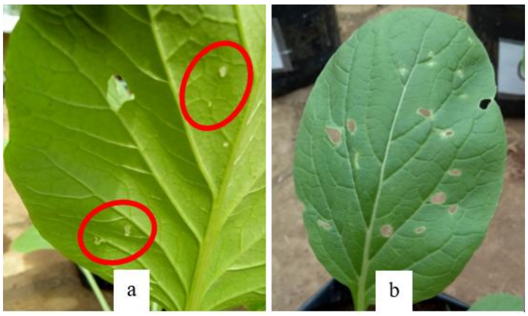

Figure 1. *Plutella xylostella* (a, red cycle) attack on choy sum caused perforated leaves (b)

Figure 2. Performance of plant treated with 1 dose of NPK + 1 dose of BF (G1) dan ¾ dose of NPK + 1 dose of BF (F1) compared to control (A1)

The shoot fresh weight of choy sum with biofertilizer only was not different with control. Plant treated with various NPK and various combination of NPK and biofertilizer increased up to 188%; which is depend on both fertilizers dose. Significant plant height increment was showed by plant treated with NPK (21.5%) and 1 dose of NPK + 1 dose of BF (28.8%).

Table 3. Effect of NPK fertilizer application combined with biofertilizer consortia on shoot fresh weight and height at 25 days after transplanting

Fertilizers Treatments	Fresh weight (g)	Plant height (cm)
A = Control (without fertilizer)	30.67 a	30.2 a
B = 1 dose of NPK	79.67 bc	36.7 b
C = 1 dose of biofertilizer (BF)	31.00 a	30.2 a
D = ¼ dose of NPK + 1 dose of BF	53.67 b	34.8 a
E = ½ dose of NPK + 1 dose of BF	72.33 ab	35.1 ab
F = ¾ dose of NPK + 1 dose of BF	87.33 c	34.4 a
G = 1 dose of NPK + 1 dose of BF	88.33 c	38.9 b
H = ¾ dose of NPK + ¾ dose of BF	85.00 c	34.7 a
I = ¾ dose of NPK + ½ dose of BF	89.67 c	35.5 ab
J = ¾ dose of NPK + ¼ dose of BF	80.33 bc	35.9 ab

Note: Values followed by the same letters were not significantly difference based on Tukey test at $p \leq 0.05$.

The bacterial population increments in certain fertilizer treatments showed that NPK fertilizer as well as biofertilizer has a role in bacterial proliferation. Application of NPK not only benefit for plant but also provide major essential nutrient for bacteria. In bacterial metabolisms, N and P are essential for enzymes synthesis and ATP production respectively [20]. Biofertilizer inoculation in this study is promising to increase both bacterial population, this finding agree with the increase on population of the Bacillus, Burkholderia, Rhizobium, Streptomyces, as well as Mycobacterium after biofertilizer application [21]. Shoot weight increment after NPK application in biofertilizer inoculation was induced by the nutrient sufficiency that support plant metabolism and hence growth. Inorganic fertilizer effect on nutrient uptake and accumulation as well as plant growth have been clear [22]. Meanwhile the role of biofertilizer used in this experiment to enhance shoot height and weight was caused by the ability of microbes in nitrogen fixing and phosphate solubilizing [1,2]. Moreover, the microbes in biofertilizer enable to produce phytohormones as described by certain research [8,9]. The current study explained the role of biofertilizer to improve plant growth when inorganic fertilizer was applied in the same time. Therefore, part of available N and P released from NPK fertilizer can be substituted by enzymatic activities of the N-fixing bacteria and P-solubilizing microbes in biofertilizer.

CONCLUSION

Liquid consortia biofertilizer composed of N-fixing bacteria and P-solubilizing microbes with or without NPK compound fertilizer did not improve the population of both bacteria compare to the control. However, recommended dose of biofertilizer combined with ¼ dose of NPK and ¾ dose of NPK has a potency to increase Azotobacter and Pseudomonas count respectively. Plants treated with recommended dose of NPK combined with recommended dose of biofertilizer has the same shoot height with plants received only 1 dose of NPK fertilizer. Higher yield demonstrated by plants treated with half and ¾ dose of NPK combined with recommended or reduced dose of biofertilizer; and plants with recommended dose of NPK and biofertilizer. The current study verified that inoculation of consortia biofertilizer to potted choy sum decreased the use of NPK up to 50%.

ACKNOWLEDGMENT

Authors thank to the Laboratory of Soil Biology Universitas Padjadjaran to provide the liquid biofertilizer.

REFERENCES

[1] S. Sivasakthi, P. Saranraj and P. Sivasakthivelan. "Biological nitrogen fixation by Azotobacter sp.—A review". *Indo Asian Journal of Multidisciplinary Research*. vol. 3, pp. 1274–1284. 2017.

[2] S.B. Sharma, R.S. Sayyed, M.H. Trivedi, and T.A. Gobi, "Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils", *Springerplus.* vol. 13, no. 2, pp. 587, 2013.

[3] A. Saeid, E. Prochownik, and J. Dobrowolska-Iwanek, "Phosphorus solubilization by Bacillus Species", *Molecules.* vol. 23, no.11, pp. 2897, 2018.

[4] N.N. Danapriatna, N. N "Penjaringan Azotobacter sp. dan Azospirillum sp dari ekosistem lahan sawah sebagai sumber isolat pupuk hayati penambat nitrogen". *Jurnal Agrotek Indonesia* vol.1, no.2, pp.115-122, 2016.

[5] S. Mahato, and A. Kafle, "Comparative study of Azotobacter with or without other fertilizers on growth and yield of wheat in Western hills of Nepal", *Annals of Agrarian Science.* vol. 16, no.3, pp. 250–256, 2018.

[6] S. Sankaralingam, S. Eswaran, B. Balakan, M. Sundaram, and T. Shanka, "Screening and growth characterization of phosphate solubilizing bacterium Pseudomonas Aeruginosa". *Advances in Environmental Biology,* vol.8, no.13, pp. 673-680, 2014.

[7] R. Hindersah, M.R. Setiawati, P. Asmiran, and B.N. Fitriatin, "Formulation of Bacillus and Azotobacter consortia in liquid cultures: Preliminary research on microbes-coated urea". *International Journal of Agricultural System,* vol.8, no.1, pp. 1-10, 2020.

[8] A. Costacurta, and J. Vanderleyden. "Synthesis of phytohormones by plant-associated bacteria". *Critical Review on Microbiology,* vol. 21, no. 1. pp. 1-18, 1995.

[9] S Pons. S. Fournier, C. Chervin, G. Bécard, S. Rochange, F. Dit, et al, "Phytohormone production by the arbuscular mycorrhizal fungus Rhizophagus irregularis". *PLoS ONE,* vol. 15, no, 10, e0240886, 2020.

[10] E. J. Rubio, M.S. Montecchia, Tosi, M. F.D. Cassán, A. Perticari, and O.S. Correa. "Genotypic characterization of Azotobacteria isolated from Argentinean soils and plant-growth-promoting traits of selected strains with prospects for biofertilizer production". *The Scientific World Journal*. 2013.

[11] A. Iqbal and S. Hasnain. "Auxin producing Pseudomonas Strains: Biological candidates to modulate the growth of Triticum aestivum beneficially". *American Journal of Plant Sciences,* vol. 4, no. 9, pp.1693-1700, 2013.

[12] H. Naseem. A. Muhammad, A.S. Muhammad, and N. Khan, "Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance". Review Journal on *Basic Microbiology*. Vol. 58, no. 12, pp.1009-1022, 2018.

[13] Y. Shabatai, "Production of exopolysaccharides by Acinetobacter strains in a controlled fed-batch fermentation process using soap stock oil (SSO) as carbon source". *International Journal of Biologi Macromolecule,* vol. 12, no. 2, pp. 145-152, 1997.

[14] A.M. Abou zied, E.H. Abd El-Zaher, H.A.Elsharquawy and T. Hammad, "Exopolysaccharides Production and

Characterization from Marine-Derived Penicillium commune KP942881.1 with Some Medical Potential Applications". *Egyptian Journal of Botany,* vol. 57, pp. 17-30. 2007.

[15] A. Rahimi, S.S. Moghaddam, M. Ghiyasi, and S. Heydarzadeh, "The influence of chemical, organic and biological fertilizers on agrobiological and antioxidant properties of syrian cephalaria (*Cephalaria syriaca L.*)". *Agriculture,* vol. 9, 122

[16] T. Biswas, and S.C Kole. "Soil organic matter and microbial role in plant productivity and soil fertility". In: Adhya, T., B. Mishra, K. Annapurna, D. Verma, D. and U. Kumar. (eds) Advances in Soil Microbiology: *Recent Trends and Future Prospects Microorganisms for Sustainability*. Vol 4. pp 219–238. Springer, Singapore. 2017. [online] https://doi.org/10.1007/978-981-10-7380-9_10

[17] D.B. Zandonadi, M.P. Santos, L. Medici and J. Silva "Action of organic matter and its fractions on vegetables physiology. *Horticultura Brasileira,* vol. 32, no, 1, pp.14-20, 2014.

[18] A. Ben-David and C.E. Davidson. "Estimation method for serial dilution experiments". *Journal of Microbiological Methods,* vol. 107, pp. 214-221, 2014.

[19] W.M. Pandeirot, N.N. Wanta dan B.A. Pinaria. "Populasi larva *Plutella xylostella Linn.* pada tanaman kubis di kelurahan Paslaten Kecamatan Tomohon Timur Kota Tomohon". *Cocos*. 6(10). 11 hal. 2015. [online] https://ejournal.unsrat.ac.id/v3/index.php/cocos/article/view/8229. [Diakses tanggal 11 Desember 2022].

[20] J.C. Pommerville "*Fundamental of Microbiology". Massachusetts: Jones & Bartlett Learning, Burlington, 2021.

[21] L. Dong, Y. Li, J. Xu, J. Yang, G. Wei, L. Shen, W. Ding and S. Chen. "Biofertilizers regulate the soil microbial community and enhance Panax ginseng yields". *China Medicine*. vol. 14, no.20, 2019.

[22] J. Kihara, M. Bekunda, V. Chimonyo, A. Kimaro, B. Kotu, S. Lyimo, and W. Mhango. *"Management of soil fertility through application of fertilizers"* in Hoeschle-Zeledon, I., J. Odhong, and M. Bekunda (eds). Sustainable Agricultural Intensification. Chapter 4, pp 48-61. CAB International. Wellingford, UK. 2022.