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Vermicompost Enhances the Effectiveness of Arbuscular Mycorrhizal Fungi, Cowpea Development and Nutrient Uptake

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Abstract

Background: Optimum plant nutrition with various organic and inorganic fertilizers such as synthetic fertilizer, compost, mycorrhiza and bacterial isolations have a pivotal role in agricultural production. This study was laid out to observe the combined impact of mycorrhiza and vermicompost treatments on plant growth and nutrient uptake of cowpea.

Methods: The experiment was laid out under controlled greenhouse conditions at Tokat Gaziosmanpaşa University in 2021. Arbuscular mycorrhizal fungi and vermicompost treatments were used in the experiment. The experiment was laid out in split-plot randomized complete design with three replications.

Result: Individual performance of vermicompost amendment was more effective on nutrient uptake and seedling growth compared with alone mycorrhizal treatment and control. However, the combined application of mycorrhiza and vermicompost exhibited a remarkable synergistic impact on each other. In particular, combined application of vermicompost and mycorrhizal fungi induced leaf phosphorus content up to 72% over control seedlings. In conclusion, vermicompost both increased effectiveness of mycorrhizal fungi and also promoted nutrient uptake, dry matter accumulation and seedling growth in cowpea.

Key words: Microbiological treatment, Organic fertilizer, Plant nutrition, Synergistic impact, *Vigna unguiculata.*

Introduction

Arbuscular mycorrhizal fungi (AMF), which has a ubiquitous distribution in known global ecosystems, uses the plant-fixed carbon which is provided by the host plant, in return, it induces plant growth by rising water and nutrients via extraradical and intraradical hyphae, and the root apoplast interface (Parniske, 2008). Indeed, while the primary beneficial role of AMF is considered as nutrient supply in particular poor soils, secondary roles of them are thought to a reduction of root invasion by soil-borne microbial plant pathogens, protection of plants from phytotoxic impacts of heavy metals, enhancing water balance under stress conditions (Abeer *et al*., 2015) and decreasing of insect herbivory (Neumann and George, 2010). Besides, to the best of our knowledge, combined application of AMF with various organic and biological amendments induces effectiveness of AMF (Cavagnaro, 2015; Sagar *et al*., 2021).

Vermicompost is an innovative technology in the sustainable agricultural perspective due to the decomposition and conversion of organic residues into rich and eco-friendly organic manure by the special earthworms. The basic difference that distinguishes vermicompost from other organic amendments is exposure to the action of some enzymes and microorganisms while passing through the digestive system of earthworm (Ravindran *et al*., 2016). Vermicompost treatment promotes germination and seedling growth (Ceritoglu *et al*., 2021), improves crop yield and quality (Joshi *et al*., 2015), and enhances soil physiological, chemical and biological properties (Arancon and Edwards, 2011), reduces pathogenic infections (Yatoo *et al*., 2021). Besides, vermicompost has also many direct and indirect effects on plants and soil such as improving soil structure, increasing organic matter and carbon content, porosity and water retention reducing bulk density, promoting microbial biomass and activity, suppressing plant diseases, and inducing various enzymatic activities, *i.e*., dehydrogenase, nitrogenase, phosphatase (Singh *et al*., 2020).

Beneficial and synergistic influences of vermicompost on the promotive effect of AMF were already stated by various researchers (Pireh *et al*., 2017). However, the information about the combined application of vermicompost and AMF in cowpea production is limited. Cowpea is a major crop for human and animal feed in particular places in which malnutrition is widely seen such as the African continent (Omomowo and Babalola, 2021). Therefore, improving cowpea growth through sustainable strategies constitutes a part of food security in the world.

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MATERIALS AND METHODS

Vermicompost, arbuscular mycorrhizal fungi and soil

Three cowpea cultivars (Akkiz, Karagöz, Karnikara) belonging *Vigna unguiculata* were used in the experiment. Cow manure-based vermicompost was supplied from the commercial company. Physio-chemical properties of vermicompost were given in Table 1. Mycorrhizal bio-material was supplied by BioGlobal commercial company. Out of experimental materials, field soil was gathered from A horizon of agricultural production area of Tokat Gaziosmanpaşa University. Experiment soil was analyzed and soil characteristic was summarized in Table 1.

Growth conditions, treatments and experimental protocol

The experiment was laid out under controlled greenhouse conditions in Tokat Gaziosmanpaşa University, Tokat, Turkey during April and March of 2021. The geographical position of the greenhouse is between 40°19′ 58″ N and 36° 28′ 25″ E. Altitude is 595 meters. Temperature and relative humidity were set as 25-27°C and 60-70% in the greenhouse, respectively. The light/dark period was 14:10 through the experiment.

Three cultivars and eight treatments including different combinations of vermicompost and AMF were used in the experiment. Treatments were T1: control (100% soil), T2: 100% soil+AMF, T3: 15% vermicompost, T4: 30% vermicompost, T5: 45% vermicompost, T6: T3+AMF, T7: T4+AMF, and T8: T5+AMF. The experiment was laid out in split-plot randomized complete design with three replications and repeated two times. Cowpea cultivars and treatments were placed in main plots and sub-plots, respectively. Seedlings were grown in viols (4.5x6 cm^2). Before starting the experiment, field soil was autoclaved at 121°C through 20 munites to avoid natural microorganisms' effect. Viols were divided into three sections, therefore, each replication was constituted of three sections. Just one cultivar was sown in each viol and different treatments were applied above mentioned.

Arbuscular mycorrhizal fungi application and experimental layout

Surface sterilization was applied to seeds with 2% of sodium hypochlorite for 5 minutes to avoid fungal and bacterial microorganisms before sowing. One seed was sown to each section of viols and irrigated by tapwater. Solutions containing 5000 ppm concentration of AMF were prepared using distilled water. The 50 ml of AMF solution per viol (~20-25 spores g^{-} soil) was injected into the rhizosphere of cowpea seedlings four days after emergence. The 50 ml of distilled water was given to each non-AMF applied viol at the same time. Seedlings were irrigated with the Hoagland nutrient solution. The EC of Hoagland solution was adjusted as 1.4 mmhos cm^{-} until seedlings achieve 14-days of age and after this period EC was set as 1.8 mmhos cm^{-}. The experiment was conducted at the end of the 40th day.

Table 1: Basic physio-chemical properties of soil and vermicompost.

	Soil	Vermicompost
pH (1:2.5)	7.86	7.8
EC (Ds m^{-})	6.69	5.0
$CaCO_3$ (%)	16.8	-
Organic matter (%)	1.21	48.95
Total N (%)	0.15	1.90
P_2O_5 (kg da^{-})	3.12	2.05
K_2P (kg da^{-})	153	0.8
Iron (ppm)	1.45	-
Zinc (ppm)	0.38	-
Humic and fulvic acid	-	20
C:N	-	14.94
Texture	Clay loam	-

(S: Soil, VC: Vermicompost, EC: Electrical conductivity, H: Humic acid, F: Fulvic acid. Field soil was slightly alkaline and saline, and limely. PH was alkaline near neutral. Field soil had low organic matter content. Total phosphorus and nitrogen were low, but, potassium was sufficient. Iron and zinc were insufficient. Cow manure-based vermicompost material had a high organic matter and humic substances. Besides, vermicompost had slightly saline and alkaline near-neutral PH. Vermicompost material was supplied from the company of Ekosol Tarim ve Hayvancilik A.S.).

Morphological observations and nutrient uptake

At the final of the experiment, ten plants were randomly selected from each viol. Plants were cut by scissors and separated root and shoot parts. Plant height (PH) was measured by a scale. Stem diameter (SD) was measured at 1 cm above the soil surface using an electronic digital caliper (Mitutoyo 500-182-30 digital caliper, Co. Ltd., Japan) to investigate seedling growth and robustness. Root fresh weight (RFW) and shoot fresh weight (SFW) were determined and samples were placed in the oven at 68°C until not any differences between the last two observations, therefore, shoot dry weight (SDW) and root dry weight (RDW) were observed. The samples were ground and sieved by 2 mm for the determination of macronutrient contents. Kjeldahl method was used to determine leaf nitrogen content (LNC) of samples (Saez-Plaza *et al*., 2013). Modified Vando-molybdate phosphoric yellow color method was used to observe leaf phosphorus content (LPC) and Leaf potassium content (LKC) was determined by flame photometric method (Jackson, 1973).

Statistical analysis

The test of Shapiro and Wilks was used to investigate the normality of the data. The results were subjected to analysis of variance using JMP (V.5.0.2) according to split-plot randomized complete design. The means were grouped by Tukey Multivariate test.

RESULTS AND DISCUSSION

According to analysis of variance, cowpea cultivars caused statistically significant differences at the level of P<0.01 in the PH, SFW, SDW, RFW and LPC while it led to significant differences ($P<0.05$) in RDW and LNC. However, cultivars did not significantly affect SD and LKC. Treatments and their interaction with cowpea cultivars caused statistically significant differences ($P<0.01$) in all traits (Table 2).

The PH depending on cultivars and treatments changed between 22.3-24.8 and 20.9-25.0, respectively. The highest pH was in Akkız and the lowest one was in Karnıkara. The highest PH depending on treatments was obtained by T8 treatment which promoted the PH up to 25% compared with control (Fig 1). All cultivars were in the same statistical group in terms of SD, however, treatments were effective. Depending on treatments, SD varied between 2.48-3.56 mm. The highest SD was observed in T8 treatment whereas the lowest one was determined by control seedlings (Fig 2). The highest SFW was obtained by T8 treatment with Karnıkara and Karagöz, respectively, following T8 treatment with Akkız. Rising vermicompost applications induced both SFW and the effectiveness of AMF (Fig 3). Differences and fluctuation between treatments were higher in SFW compared with SDW due to holding water in plant metabolism. The T8 treatment led to observing the highest dry matter accumulation in shoots. However, T8 treatment promoted dry matter accumulation over control up to 31.8%, 28.0% and 18.2% in Karnikara, Akkiz and Karagöz, respectively. The effectiveness of AMF in these promotive impacts was about 5% in all cultivars (Fig 4). RFW and RDW were similarly affected by treatments and the highest RFW

Table 2: Analysis of variance belonging to investigated morphological and chemical characteristics depending on cowpea cultivars and treatments including vermicompost and arbuscular mycorrhizal fungi.

Source of variation		PH			SFW		SDW	
	DF	MS	F prob.		MS	F prob.	MS	F prob.
Cultivars (C)	2	48.92	**		14.49	**	0.208	**
Treatments (T)	7	21.56	**		89.73	**	0.302	**
C × T	14	1.82	**		1.45	**	0.014	**
		RFW			RDW		SD	
	DF	MS	F prob.		MS	F prob.	MS	F prob.
Cultivars (C)	2	2.58	**		0.205	*	0.00003	ns
Treatments (T)	7	13.62	**		0.259	**	1.60255	**
C × T	14	0.15	**		0.006	**	0.00001	**
		LNC			LPC		LKC	
	DF	MS	F prob.		MS	F prob.	MS	F prob.
Cultivars (C)	2	0.057	*		0.0108	**	0.0013	ns
Treatments (T)	7	0.383	**		0.0362	**	0.0559	**
C × T	14	0.014	**		0.0007	**	0.0029	**

PH: Plant height, SFW: Seedling fresh weight, SDW: Seedling dry weight, RFW: Root fresh weight, RDW: Root dry weight, SD: Stem diameter, LNC: Leaf nitrogen content, LPC: Leaf phosphorus content, LKC: Leaf potassium content, DF: Degree of freedom, MS: Mean of square, **: $P<0.01$, *: $P<0.05$, ns: No significant difference.

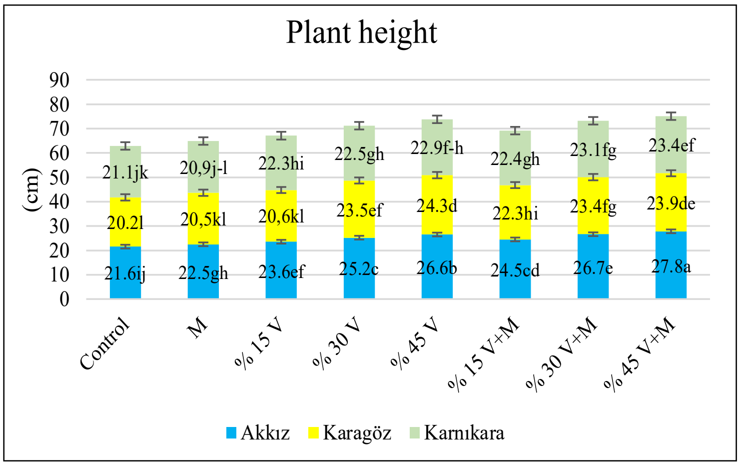

Fig 1: Influence of vermicompost and mycorrhizal applications on plant height of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at $p<0.01$).

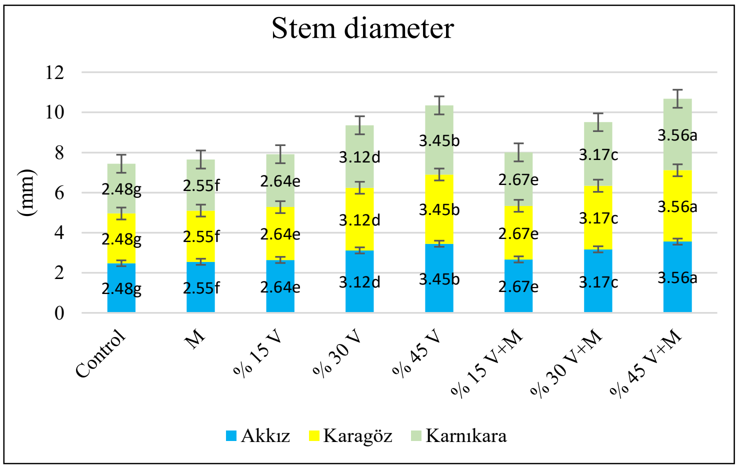

Fig 2: Influence of vermicompost and mycorrhizal applications on stem diameter of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at p<0.01).

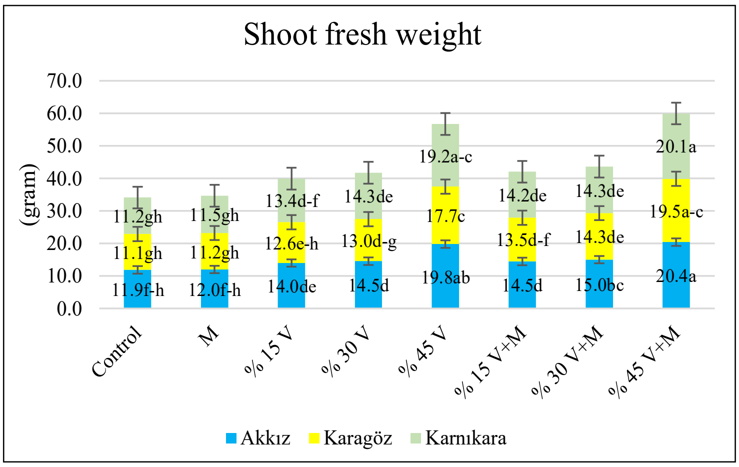

Fig 3: Influence of vermicompost and mycorrhizal applications on shoot fresh weight of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at p<0.01).

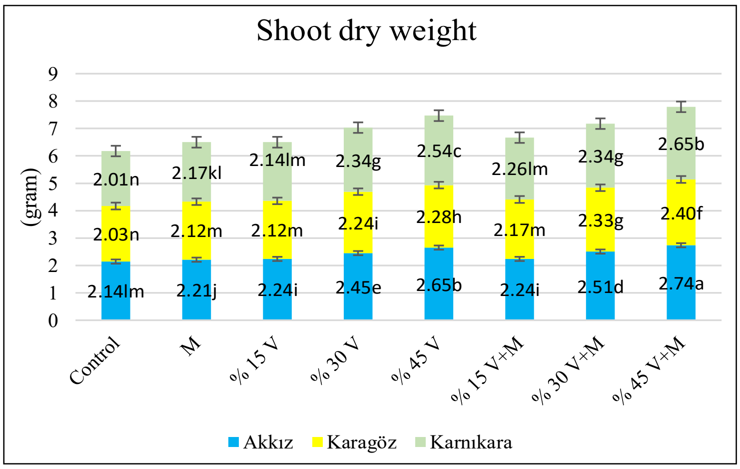

Fig 4: Influence of vermicompost and mycorrhizal applications on shoot dry weight of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at p<0.01).

and RDW were obtained by the T8 treatment in Akkiz (Fig 5 and 6). The highest LNC was determined with T8 treatment in Akkiz while the lowest one was observed in control of Karnikara seedlings (Fig 7). In particular, the effectiveness of 45% vermicompost and its synergistic relationship with nitrogen uptake was remarkable since the T5 and T8 treatments increased the LNC compared with control up to 11.7% and 28.5%, respectively (Fig 10). The consortium of vermicompost and the mycorrhizal was more effective on phosphorus uptake than nitrogen (Fig 8). Promoting rate of the LPC belonging to cultivars by treatments varied between 4-72%, and the highest increment was observed in T8 treatment. Although individual AMF treatment was not very effective on P uptake, it has a remarkable impact when used with vermicompost (Fig 10). The highest LKT (1.69%) was determined with T8 treatment in Karagöz while the lowest LKT was observed with control seedlings of Karnýkara. The LKC varied between 1.41-1.69% depending on cultivars and treatments. Treatments induced potassium uptake by 2.1-15.3%. The highest increment was determined with T7 treatment following T8 (Fig 9).

The AMF facilitates nutrient uptake under optimum and stress conditions. AMF treatment increased PH, SFW and SDW, induced dry matter accumulation in all parts and enhanced the LNC, LPC and LKC in seedlings, therefore, the findings are in agreement with previous experiments (Etesami *et al*., 2021). On the whole, the primary contribution of AMF is considered as increasing the supply of nutrients to plants, in particular, ionic forms of poor-mobility ones such as ammonium, phosphate, copper and zinc (Barea *et al*., 2005). Neuman and George (2010) indicated that AMF both directly contributes to nutrient uptake by symbiotic pathway, and also indirectly influences it via alteration of the root system and functioning and soil properties of mycorrhizosphere. It is considered that AMF hyphae may be able to achieve in smaller soil pores due to their thin

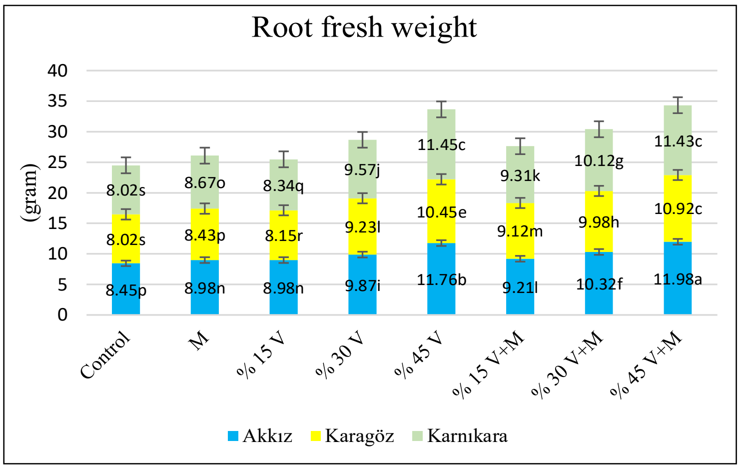

Fig 5: Influence of vermicompost and mycorrhizal applications on root fresh weight of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at p<0.01).

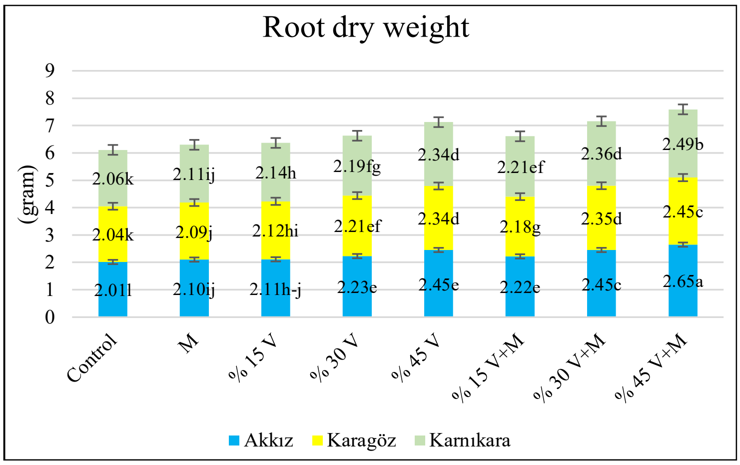

Fig 6: Influence of vermicompost and mycorrhizal applications on root dry weight of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at p<0.01 or 0.05).

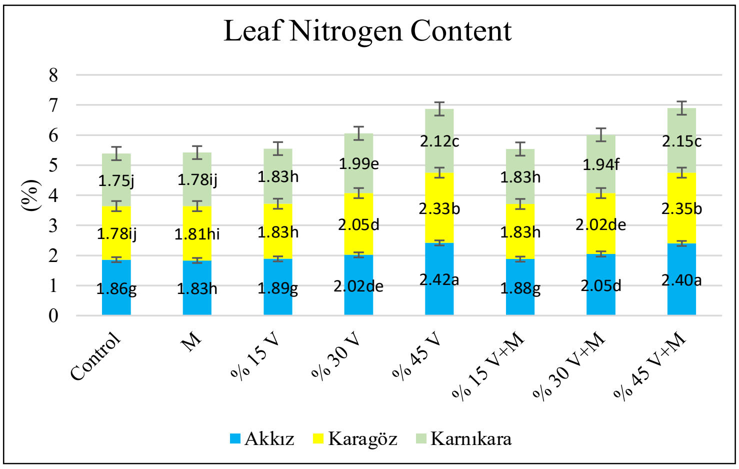

Fig 7: Influence of vermicompost and mycorrhizal applications on the leaf nitrogen content of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at $p<0.01$ or 0.05).

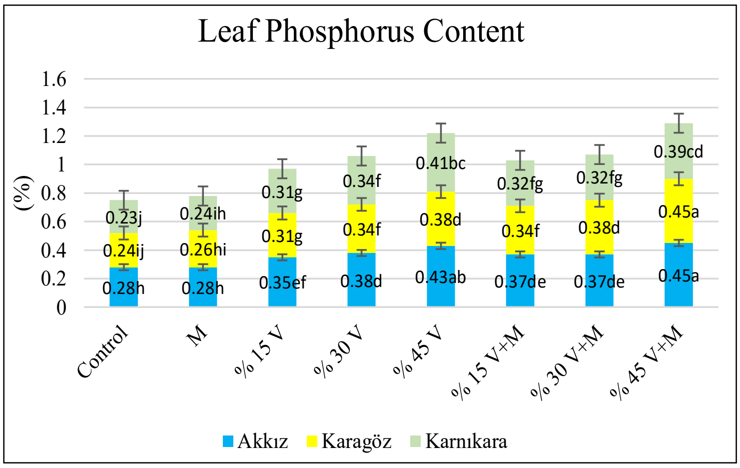

Fig 8: Influence of vermicompost and mycorrhizal applications on the leaf phosphorus content of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at $p<0.01$).

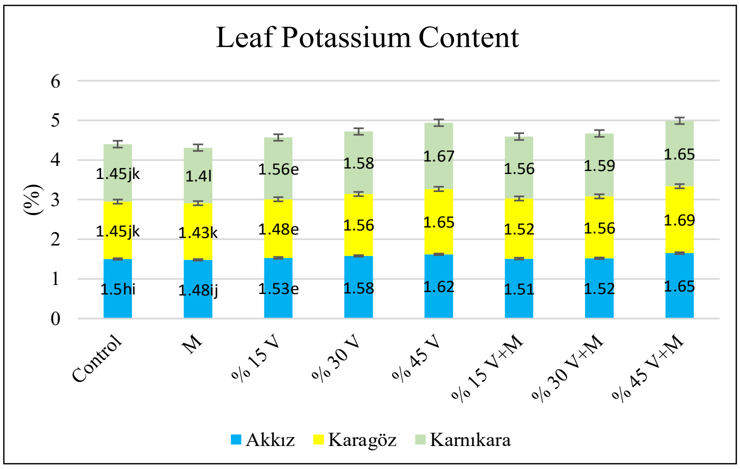

Fig 9: Influence of vermicompost and mycorrhizal applications on the leaf potassium content of three cowpea cultivars (V: Vermicompost, M: Mycorrhizal application. Means and standard error bars were given in the graph. Different letters denote statistically significant differences at $p<0.01$).

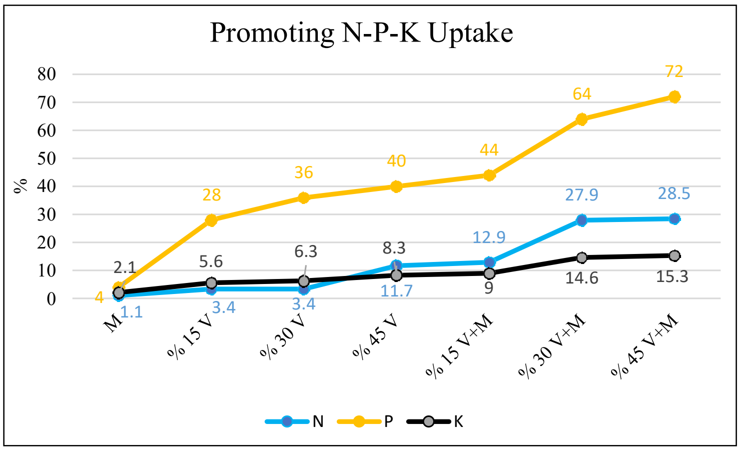

Fig 10: Alterations in nutrient uptake depending on the individual and combined application of vermicompost and arbuscular mycorrhizal fungi compared with non-amended control plants (V: Vermicompost, M: Mycorrhizal application).

diameter, thereby, they can exhibit better competition against soil microbes for nutrient resources. Moreover, AMF might enhance the chemical mobilization of nutrients via activation of inorganic phosphate and ammonium transporters, as well as, plant morphological growth (Banasiak *et al*., 2021). Thus, AMF applications promoted nitrogen, phosphorus and potassium uptake and transportation to aboveground parts of plants and enhanced morphological growth of roots and shoots in all cultivars. Besides, individual and combined application of vermicompost caused a remarkable promotion of nutrient uptake and seedling growth. It is considered to be caused by alteration of soil carbon and nutrient pools depending on vermicompost doses. Sarma *et al*. (2018) denoted that vermicompost application led to increasing soil organic carbon, C-mineralization and soil respiration. So, these changes promote C transportation to AMF, nutrient uptake by hype and transmit to plant roots and increase seedling growth and development. Ceritoglu *et al*. (2018) pointed out that vermicompost enhances soil nutrient pool, promotes microbial activity and improves physiological properties. This experiment also presented that N-P-K uptake gradually increased by individual application of vermicompost and continued to increase by the synergistic influence of AMF. In particular, the LPC increased from 28% to 40% depending on individual vermicompost doses, however, it achieved 72% with AMF treatment. The findings are in agreement with Etesami *et al*. (2021) who reviewed the effectiveness of AMF on phosphorus uptake. Muthukumar and Udaiyan (2002) stated that organic manures have a promotive impact on the effectiveness of AMF and soil N-P-C pool and more, there are strong positive correlations between nutrient uptake, plant growth and yield depending on mycorrhizal and organic applications.

Although AMF treatment promoted nutrient uptake and plant development compared with control seedlings, its alone performance was not satisfactory in the experiment. The reason for this situation is guessed to be caused by the photosynthetic pathway of cowpea (C3). Wilson and Hartnett (1998) stated that plants containing the C3 photosynthetic pathway such as legumes have low mycorrhizal response over other species including the C4 pathway. In conclusion, vermicompost application is an eco-friendly and effective scenario for nutrient uptake and seedling growth in cowpea cultivation and more, combined application of vermicompost with AMF has a noteworthy synergistic influence on each other.

CONCLUSION

although individual AMF application had a positive impact compared with control seedlings, individual performance of vermicompost was more effective on investigated characteristics. Moreover, combined application of them exhibited a remarkable performance and increasing concentration of vermicompost induced AMF effectiveness, nutrient uptake and seedling growth. In particular, phosphorus uptake was noteworthy induced by combined AMF and 45% vermicompost treatment. The AMF inoculation with 45% vermicompost amendment highly induced seedling growth and nutrient uptake in cowpea plants.

Conflict of interest: None.

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