Humic Acid for Reducing Carbofuran Content of Rice Grown in Carbofuran-Contaminated Soil

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ABSTRACT

Carbofuran insecticide widely used in rice production. The carbofuran residue in agricultural threatens wide life and human health since its active ingredient carbamate is toxic. Humic acid (HA) amendment is an effective way to minimize the residue in soil since HA can adsorb the organic pollutant and induce growth of fungus that degrade the carbofuran. The objective of this research was to observe the influence of HA on the carbofuran residues in soil, straw, and rice; as well as yield of paddy (Oryza sativa L.) grown in carbofuran contaminated potted soil. The level of carbofuran residue in soil before experiment was 10 mg kg^{-1}. The experiment was setup in Randomized Block Design to test various HAs extracted from municipal waste, cow manure, and peat. The results indicated that humic acid didn’t affect plant growth and yield but decreased the carbofuran residue in straw and unhusked rice grain. Rhizosphere of rice treated with HA showed higher fungal count than control. This experiment suggests that HA were effectively used to control the carbofuran residue in soil and rice grown in low carbofuran-contamination agricultural soil.

Keywords: Carbofuran residue, soil acidity, straw, unhusked grain, yield

INTRODUCTION

Rice is important staple food in southeast Asia, therefore, maintaining rice productivity is needed. Nowadays, farmers depend on chemical pesticide even though environmental-friendly cultural practice of rice production is suggested. Carbofuran is one of the active ingredients of carbofuran pesticide widely used in rice fields to control the insects, such as brown planthoppers and rice stem borer. Carbofuran is a synthetic substance of Carbamate (2,3-dihydro-2,2-dimethyl-7-benzofuranoyl N-methylcarbamate) that can also function as a nematicide and acaricide. Carbofuran is a toxic compound that is highly soluble in water and relatively mobile in the soil (Singh and Srivastava, 2009). As a result, carbofuran residues can possibly contaminate soil, plants, ground water, and river.

Humic substances are a heterogeneous mixture of different organic compounds synthesized from organic matter and decomposed by soil microbes. The humic acid (HA) is a type of humic substance that have colloids nature and their characteristics in solution is similar to the micellar aggregates (Yan *et a*l., 2021). It is well known that HA have high specific surface and negative charge with moderately persistence in soil. In solution, HS have a carboxyl group (-COOH) and a hydroxyl group (-OH) that can form the complexes with heavy metals (Sudiono *et al*., 2017) or organic pollutant (Chianese *et al*., 2020) such as pesticide.

The adsorption of pesticide residues by HA in soil limits the mobilization of pesticides and reduces the level of toxicity to living things. The carbofuran adsorption capacity of the soils was low and dependent on organic carbon contents that plays a key role in the irreversibility of carbofuran adsorption (Shaheen *et al*., 2019). The availability of carbofuran in soil depends on the mineralization rate that increases with rise in temperature and moisture content (Benicha *et al*., 2013).

Application of HA into the soil will provide multiple benefits; increased carbofuran adsorption and growth induction of carbofuran-degrading soil fungi. Enzymatic degradation of carbofuran as a carbon source for heterotroph microbes in through hydrolysis and oxidation soil (Mishra *et al*., 2023). The fungus is capable of utilizing carbofuran as a sole carbon and energy source (Devi and Iyer, 2016). Fungi *Trametes versicolor* and *Phanerochaete chrysosporium* showed the highest HA removal efficiency, reaching about 80%; Laccase and manganese peroxidase extracellular enzymes production of both fungi is induced by low nitrogen in soil (Zahmatkesh *et al*., 2016). The genus *Gliocladium* has the highest ability in carbofuran degrading compared to bacterial isolates (Devi and Iyer, 2016). Antagonistic Trichoderma spp. were found to degrade 200 mg/kg carbofuran in soil as a sole carbon to 3-ketocarbofuran within 14 days (Afify *et al*., 2012).

The accumulation of carbofuran residue in edible parts of food crops is reported (Kumar *et al*., 2019; Lv *et al*., 2022). Amount of carbamates pesticide residue in grains was 0.25 mg/kg; the lowest compared to vegetable and fruits (Fatunsin *et al*., 2020). By using 420 g carbamate for 100 kg rice seeds, the carbofuran and 3-hydroxy carbofuran in brown rice were less than 0.05 mg/kg which is lower than MRL for carbofuran, 0.1 mg/kg (Zhang *et al*., 2016). The minimum limit for carbofuran residue for rice according to the Indonesian National Standard is also 0.1 mg/kg.

The negative impact of carbofuran residue can be harmful to humans, particularly when it accumulates in edible part of food crops. A pot experiment was conducted to observe the effect of various HAs on the carbofuran residue in soil and rice crops as well as yield of rice grown in carbofuran-contaminated soil. Moreover, this experiment was aimed to verify the change of fungal population in carbofuran-contaminated soil after HA application.

MATERIALS AND METHOD

The experiment has been carried out in the greenhouse of Agricultural Environment Research Centre in Pati, Indonesia, located about of 7 m above sea level. The potted soil was Inceptisols with acidic reaction (pH 5.2) and clayed texture. Soil has only 1.46 % organic carbon but average in nitrogen (0.25%). The soil was contaminated with 10 mg/kg carbofuran before experimental setup. The HA were extracted from local organic municipal waste (MW), cow manure (CM) and peat (P); the concentration of HA in MW, CM and P were found about 0.6%, 3% and 2% respectively.

Experimental Setup

The design of greenhouse experiment was Randomized Block Design to test three HA treatments; 0.225 g/pot municipal waste HA; 1.125 g/pot cow manure HA; 0.750 g/pot peat HA; and one control treatment (plants without HA). All treatment was replicated six times. The HA doses of each treatment were calculated based on the recovered concentration of HA in each organic matter described above; and weight of soil in a pot (7.5 kg) as well as rate of HA in a hectare (10 ton/ha).

Rice cv Ciherang were grown in black polyethylene pot amounted to 7.5 kg and soil. Before experiment the soil was air dried without direct sunshine, then the potted soils were saturated with water in order to obtain 5-cm standing water. All potted soil were stored in the green house and the standing water was kept about 5 cm by adding water if necessary. Seven days later, 10 mg/kg carbofuran is mixed evenly with soil and incubated for another seven days. The HA was incorporated into the soil evenly at a depth of about 15 cm, then soils were incubated for seven days. After incubation, 21-day old rice seedling was transplanted. In each pot, two seedlings were grown. Each plant in a pot were treated with 0.38 g Urea, 0.47 g SP-36, and 0.13 g KCI, which is equivalent to the recommended doses of 100 kg/ha N, 125 kg/ha P2O5 and 33.33 kg/ha K2O. Fertilizer was put in a 10-cm depth hole at a distance of 5 cm from the rice stalks. Urea and KCI were applied twice at planting time and 21 DAP, while SP-36 is given once at the planting time. The puddle height was reduced up to 2 cm when fertilizing the paddy and increased up to 5 cm at tillering phase. Plants were irrigated by distilled water which is free from any metals and pesticide residues. Irrigation was carried out every day until 56 days after planting (DAP) and twice a day until 70 DAP. All plants were maintained in the greenhouse for 100 DAP.

Parameters and Statistical Analysis

Plant height and number of rice tillers were measured once a week from 7 to 56 DAP. Soil acidity was measured at the time of planting, as well as 60 and 100 DAP. The carbofuran residues in the soil were analyzed at planting time, 60 DAP and 100 DAP; in straw at 60 DAP and 100 DAP, and in rice grain at 100 DAP. The dry weight of unhusked grain was measured from grain with a moisture content of 15%.

Carbofuran derivatization from soil as well as plant was carried out by florisil chromatography column with sodium sulfate anhydrate, and n-hexane as the eluent. All data were subjected to Analysis of Variance (ANOVA; $p \leq 0.05$). If the parameters were influenced significantly by the humic acid treatment, then the Duncan Multiple Range Test were performed at $p \leq 0.05$.

RESULTS AND DISCUSSION

Soil Acidity

Soil reaction (pH) before experiment was acid (pH 5.2), but the pH increased during the rice growth in potted soil (Figure 1). Rice seedlings were transplanted 7 days after HA application in contaminated soil. The soil pH measured at planting day increased to slightly neutral that induce N, P and K availability in soil. At the end of experiment the soil reaction became neutral. The results found that the pH was not significantly different after various HAs amendment at $p \leq 0.05$.

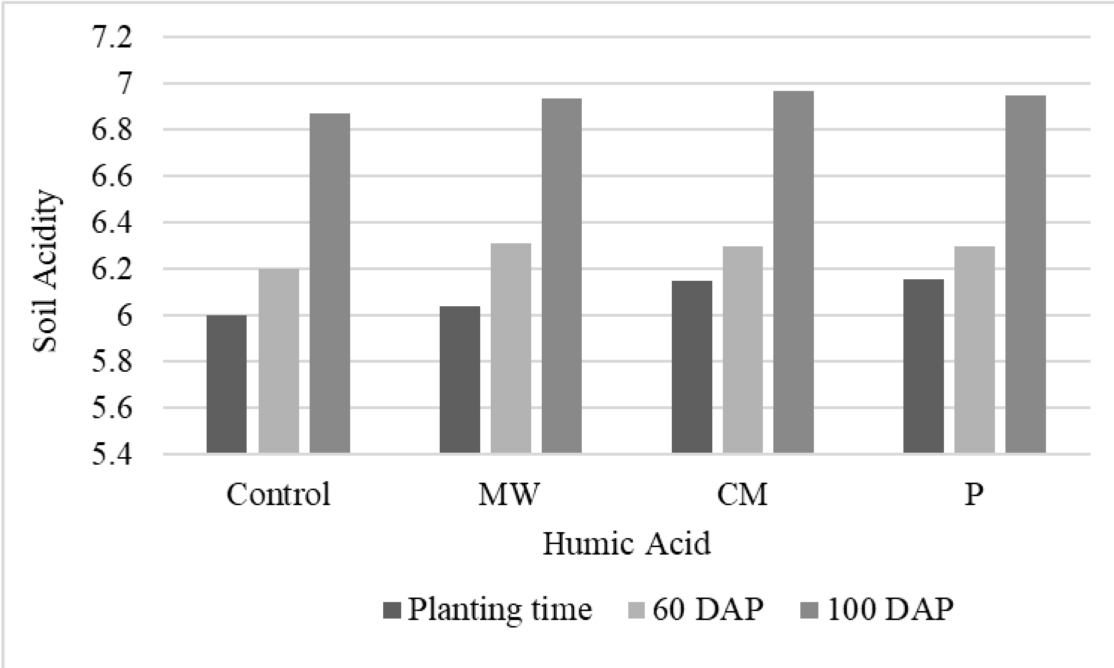

Figure 1. The acidity of soil contaminated by carbofuran after application of HA extracted from municipal waste (WP), cow manure (CM), and peat (P).

Puddling always resulted in the change of soil pH to neutral. This result is in accordance with the study of Ding *et al.* (2019) which states that during the flooding period, the soil acidity increased to approximately 7.0, whereas initial soil acidity is lower than 6.5 or higher than 6.5. In flooded soil, the changes of pH were reversible, so the pH will be decreased when soil moisture content is decreased. The opposite pattern was demonstrated by alkaline soil. This pH alteration benefits rice growth due to mobilization of phosphate and potassium in slightly neutral to neutral soil compared to acid soil (Reitsma *et al*., 2011). Increased in soil pH benefits non-rice crops grown after the rice harvest. However, the decrease in pH due to aerobic conditions will take place.

Plant height

Based on ANOVA with $p \leq 0{,}05$, the application of various HA did not affect plant height (Figure 2). In this pot experiment, carbofuran application did not affect plant performance. therefore, plants have not responded to HA application.

The HA is well known as organic soil conditioner instead of soil fertilizer. The prominent role of soil conditioner is to improve soil condition mainly soil physics to become suitable for plant growth (EI-Dolify *et al*., 2016). Inceptisols used in this experiment have a good soil structure so that the effect of HA on plant growth was not prominent. Carbofuran contamination in this experiment possibly did not reach the minimum threshold to decrease plant growth; possibly due to the adsorption of carbamate residues by clayed soil used in this pot trial (Bansal *et al*., 2014).

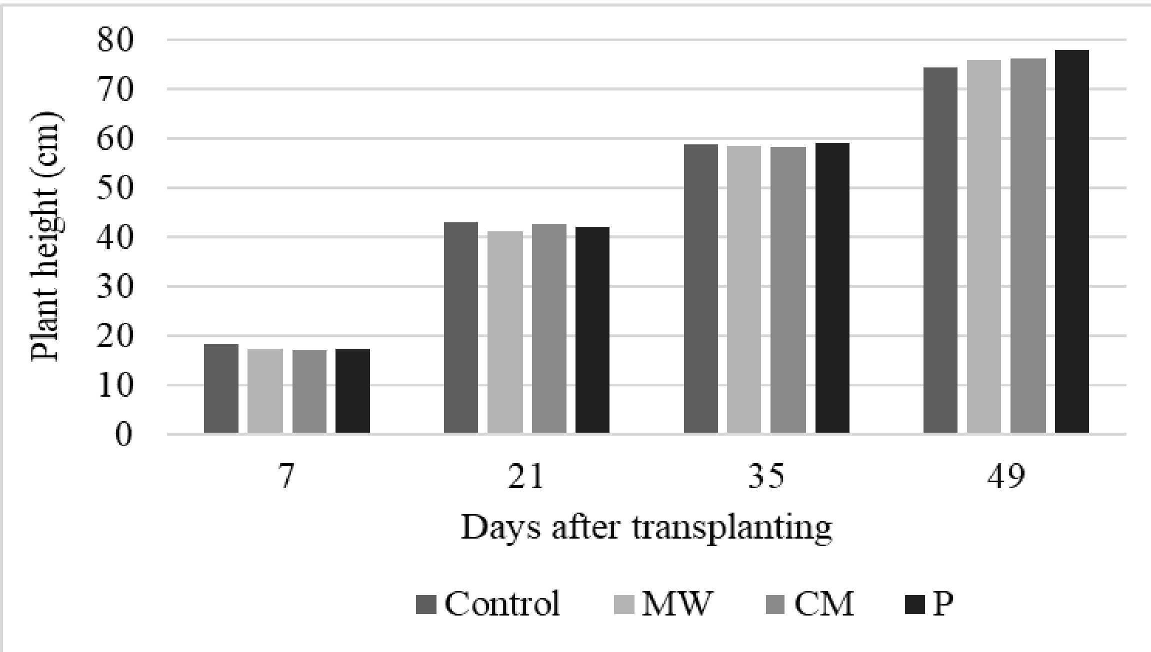

Figure 2. Plant height of rice grown in carbofiuran-contaminated soil after application of HA extracted from municipal waste (MW), cow manure (CM) and peat (P).

Carbofuran residue in soil and rice plant

Statistical analysis with $p \leq 0.05$ verified that HA that was amended 7 days before planting did not affect its residue in soil at planting time and 60 DAP. After contamination, the concentration of carbofuran was 10 mg/kg; the result verified that carbofuran residue in soil was clearly decreased after planting the rice. However, the carbofuran residue in soil was significantly reduced by HA application (Table 1). The lowest residue at 60 DAP was found in soil that treated with HA extracted from organic municipal waste. Nonetheless at 100 DAP (harvest time), all HA application decreased the carbofuran residue.

Table 1. Carbofuran residue in soil contaminated by carbofuran after humic acid application

Humic Acid Treatments	Carbofuran in soil (mg kg^{-1})		
	Planting time	60 DAP	100 DAP
Control	3.69 a	2.57 a	2.00 a
MW-humic acid	3.28 a	0.97 b	0.06 b
CM-humic acid	3.18 a	0.82 c	0.06 b
P-humic acid	4.49 a	1.25 b	0.07 b

Numbers followed by the same letters were not significantly different based on DMR test at $p \leq 0.05$. MW municipal waste, CM cow manure, P peat, DAP days after planting.

Based on ANOVA, the HA application decreased the carbofuran residue level in straw as well as unhusked grain. However, the residue level of plants treated with any humic acid were not different (Table 2). The HA-treated plants produced unhusked rice grain with lower carbofuran residue compared to the control plants, but all plants with HA had similar residue level. In Indonesia, the minimum concentration of carbofuran residues in the straw is not regulated, but commercialized husked rice should have maximal carbofuran level of 0.1 mg/kg. This experiment showed that HA treated rice contained 87.5% - 95.8% less carbofuran than control

This experiment showed that HA-treated rice contained 87.5% - 95.8% less carbofuran than control. The residual carbofuran in soil and rice of this pot experiment was higher than in farming area since the soil is purposively contaminated by pesticide. However, Chansuvarn and Chansuvarn (2018) found that the range carbofuran residue in uncontaminated soil and rice grain is ND-0.0512 µg/kg and ND-0.0865 µg/kg for soil and rice grain, respectively.

Table 2. Carbofuran residue in straw and rice grown in soil contaminated by carbofuran after various humic acid treatments

Humic Acid Treatments*	Carbofuran in straw (mg kg^{-1})		Carbofuran in rice
	60 DAP	100 DAP	(mg kg^{-1})
Control	2.53 a	1.88 a	1.45 a

MW-humic acid	0.85 b	0.56 b	0.18 b
CM-humic acid	0.88 b	0.56 b	0.06 b
P-humic acid	1.16 b	0.30 b	0.08 b

Numbers followed by the same letters were not significantly different based on DMR test at $p \leq 0.05$. MW municipal waste, CM cow manure, P peat, DAP days after planting

Unhusked Grain Weight

Despite higher content of residue in control plants, the unhusked grain dry weight was not significantly different at $p \leq 0.05$ (Figure 3). Grain yield per clump of control plant was 27.25, whereas average grain weight of HA-treated plants was about 26.72 g, only 1.9 % lower than control. The grain yield of the rice grown in carbofuran-contaminated soil was equal to rice cv Ciherang productivity, about 7 t/ha. This finding showed that in potted soil, rice cv Ciherang could withstand the slight abiotic stress caused by carbofuran contamination.

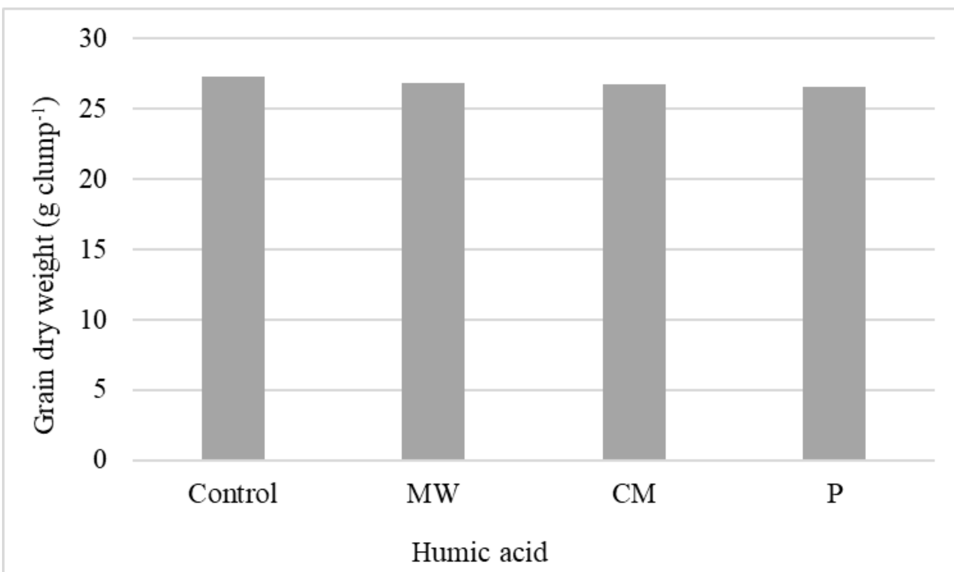

Figure 3. Yield of paddy grown in carbouran-contaminated soil after application of HA extracted from municipal waste (MW), cow manure (CM) and peat (P).

Fungal Population

Figure 4 verified that total-fungal population in the rice rhizosphere was higher than in the bulk soil. The rhizosphere is the region of soil is in vicinity of roots where microbes and roots develop intensive interaction; microbial number and type as well as soil chemistry is influenced by roots exudates (Velmourougane *et al*., 2017). For fungal growth, the root exudates that commonly contain high amount of carbohydrate is prominent as carbon and energy source during heterotrophic metabolisms (Devi and Iyer, 2016). Fungi is heterotroph which dominantly decompose new-amendment organic matter in soil and contribute to soil organic carbon stabilization (Li *et al*., 2015). Increased fungal population in the rhizosphere might be another mechanism to degrade the carbofuran as reported by some researchers (Devi and Iyer, 2016; Afify *et al*., 2012; Zahmatkesh *et al*., 2016).

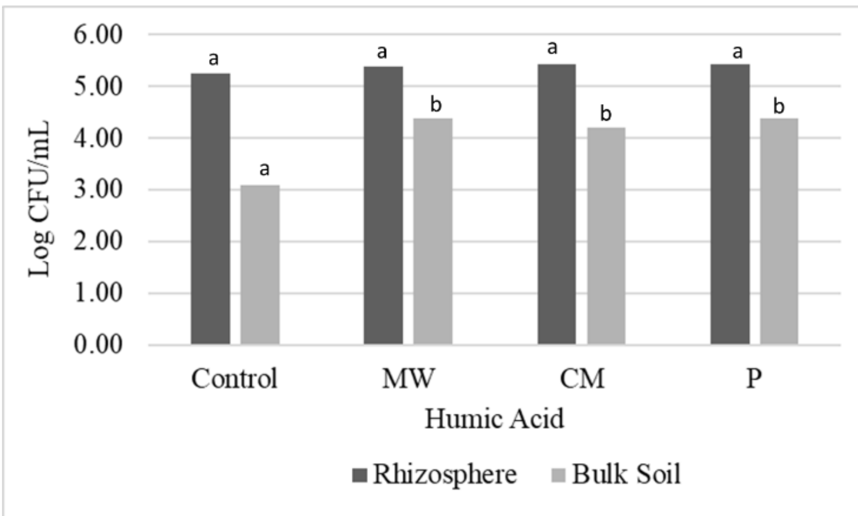

Figure 4. Fungal count in the rhizosphere and bulk soil of rice grown in carbofuran-contaminated potted soil after application of HA extracted from municipal waste (B), cow manure (CM) and peat (P). Columns with the same letters above were not significantly different based on DMR test at $p \leq 0.05$

The results found that HA application increased fungal population in the rhizosphere but not in the bulk soil. The fungal increment might be caused by the increased amount of root exudates and/or root biomass. Nonetheless, both parameters didn’t measure in this experiment. Higher fungal population in the rice rhizosphere is similar with the finding reported by other research (Banaay *et al*., 2013) for fungal count increment in the rice rhizosphere after organic matter amendment.

CONCLUSION

The pot experiment verified that plant growth and yield of rice of HA-treated and untreated pot was comparable rice growth didn’t affect by relatively low carbofuran. Application of various HAs extracted from different organic matter significantly decreased the residual carbofuran in soil as well as in straw and unhusked grain of rice grown in carbofuran-contaminated soil. Residual carbofuran of unhusked grain of HA-treated rice was 0.06-0.18 mg/kg; lower than minimal carbofuran residue (0.1 mg/kg) recommended by Indonesian Government. This founding suggest that HA enable to reduce carbofuran residue in soil.

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