UNIVERSITI PUTRA MALAYSIA

THE PHYTOTOXICITY OF PALM OIL MILL EFFLUENT, ITS DEGRADATION AND EFFECT ON PLANT GROWTH

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By

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The presence of abundant agricultural residues in Malaysia prompted the need to utilize these wastes to overcome environmental pollution. A large portion of these wastes comes from the oil palm effluent. Palm oil meal (POMeal) has been used as an organic fertilizer to increase growth and yield of several crops. However, application of raw or undecomposed POMeal can also be detrimental to the growth of certain plants. In view of this, the project conducted aimed to study the following aspects: the inhibitory effect of different levels of raw POMeal on growth of vegetables, the effect of decomposition on POMeal phytotoxicity and the degradation of *p*-coumaric and vanillic acids by *Pseudomonas* sp. and *Penicillium* sp.. In this

study the water-soluble phytotoxic compounds present in raw POMeal will be identified.

Results of the glasshouse experiment showed that growth of tomato and spinach was strongly affected by the type (raw or decomposed) and amount of POMeal applied. Growth of both plants was inhibited by application of > 1% raw POMeal. In contrast, application of 1% - 21% decomposed POMeal increased the plant's growth, with maximum dry matter production at 6% POMeal. Increase in shoot dry weight of tomato and spinach given decomposed POMeal was 7 and 178 times respectively. Increase in root dry weights was 1.6 and 62 times respectively, compared to plants given raw POMeal. Soil N, P and K contents, pH and electrical conductivity also increase with increase in POMeal levels. The phytotoxicity of raw POMeal was reduced when POMeal was decomposed for > 4 weeks.

Studies done on POMeal extract showed that the highest inhibitory activity was obtained from the diethyl ether fraction. Further separation of the extract resulted in a single fraction which comprise mixtures of *p*-coumaric and vanillic acids in the ratio of 1:9.

Several species of bacteria and fungi capable of utilising phenolic acids were isolated from sandy tailing soil and POMeal. Two of the isolates, *Pseudomonas* sp. and *Penicillium* sp. were found to be capable of utilising 500 μg ml^{-1} vanillic and *p*-coumaric acids for growth. Bacterial growth was maximum after 28 h with biomass production of 7.1 mg per 50 ml in vanillic acid and 7.3 mg per 50 ml *p*-coumaric acid. Growth of fungi was maximum after three days with biomass production of 14.5 mg per 50 ml *p*-coumaric and 11.7 mg per 50 ml vanillic acids. Utilization of the phenolic acids was faster by the *Pseudomonas* sp. P1 than *Penicillium* sp. F1

Results from pot trials showed that POMeal phytotoxicity can be greatly reduced by extending the decomposition period of POMeal. Maximum growth of spinach occurred on POMeal decomposed for ≥ 4 weeks. The increase in decomposition period also increased soil microbial population and mineralization of nitrogen. However, inoculation with pure microbial isolates had no significant effect in reducing the phytotoxicity present in POMeal.

Abstrak dissertasi yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah.

KEFITOTOKSIKAN SISA KILANG KELAPA SAWIT, PENGURAIAN DAN KESANNYA TERHADAP TUMBESARAN TANAMAN

Oleh

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Kehadiran sisa buangan pertanian yang banyak telah menimbulkan kesedaran untuk menggunakan bahan ini bagi menangani masalah pencemaran alam sekitar. Sebahagian besar sisa ini datang dari kilang kelapa sawit. 'Palm Oil Meal' (POMeal) telah digunakan sebagai baja organik bagi meningkatkan pertumbuhan dan hasil beberapa jenis tanaman. Walaupun demikian, penggunaan POMeal mentah atau yang kurang sempurna direput boleh mendatangkan kesan buruk kepada pertumbuhan tanaman tertentu. Berdasarkan masalah ini, kajian telah dijalankan untuk mengkaji aspek-aspek berikut: kesan perencatan POMeal berbeza terhadap pertumbuhan sayuran, kesan penguraian terhadap kefitotoksikan POMeal, dan

degradasi asid *p*-kumarik dan vanilik oleh spesis *Pseudomonas* dan *Penicillium*. Dalam mengendalikan kajian ini, sebatian fitotoksin larut air dari POMeal mentah akan dikenalpasti.

Keputusan kajian rumahkaca menunjukkan pertumbuhan tomato dan bayam dipengaruhi oleh jenis POMeal (mentah atau reput) dan kadar POMeal yang digunakan. Tumbesaran kedua-dua sayuran terencat dengan pemberian > 1% POMeal mentah. Sebaliknya pemberian 1% - 21% POMeal yang telah reput, didapati mampu meningkatkan tumbesaran tanaman dengan penghasilan bahan kering maksimum pada paras 6%. Berat kering pucuk tomato dan bayam yang diberi POMeal reput meningkat 7 dan 178 kali berturutan, manakala berat kering akar meningkat 1.6 dan 62 kali berturutan berbanding tanaman pada POMeal mentah. Kandungan N, P dan K tanah serta pH dan konduktiviti elektrik tanah juga meningkat dengan peningkatan paras POMeal. Kesan fitotoksik POMeal mentah berkurangan apabila POMeal telah mengurai > 4 minggu.

Kajian ke atas ekstrak POMeal menunjukkan aktiviti perencatan tertinggi diperlihatkan oleh bahagian dietil eter. Pemisahan lanjutan ke atas bahagian eter memberikan satu pecahan terdiri daripada campuran asid *p*-kumarik dan vanilik dalam nisbah 1:9.

Beberapa spesis bakteria dan kulat berupaya menggunakan asid fenolik telah dipencilkan daripada tanah pasir bekas lombong dan POMeal. Dua daripada pencilan ini, spesis *Pseudomonas* dan *Penicillium* didapati berupaya menggunakan asid vanilik dan *p*-kumarik pada kepekatan 500 μg per ml untuk hidup. Pertumbuhan bakteria adalah maksimum selepas 28 jam eraman dengan hasil biojisim 7.1 mg per 50 ml asid vanilik dan 7.3 mg per 50ml asid *p*-kumarik. Pertumbuhan kulat adalah maksimum selepas 3 hari eraman dengan hasil biojisim 14.5 mg per 50 ml asid *p*-kumarik dan 11.5 mg per 50ml asid vanilik. Penguraian asid fenolik adalah lebih cepat dilakukan oleh spesis *Pseudomonas* P1 berbanding spesis *Penicillium* F1.

Keputusan daripada kajian berpasu menunjukkan pengurangan kefitotoksikan POMeal dengan memanjangkan jangkamasa penguraian POMeal. Pertumbuhan maksimum pokok bayam berlaku pada POMeal yang telah mengurai ≥ 4 minggu. Pemanjangan proses penguraian juga meningkatkan populasi mikrob tanah dan pemineralan nitrogen. Namun demikian penginokulatan dengan pencilan mikrob tulen tidak berupaya mengurangkan kefitotoksikan POMeal dengan bererti.

CHAPTER ONE

INTRODUCTION

The maintenance of soil organic matter is an important criterion in sustaining the productivity of soils in the tropic. In Malaysia, the organic material in the top soil is rapidly being decomposed and the soils are generally heavily leached, acidic and low in nutrients. These together with the overall mismanagement of soil lead to rapid decline in soil productivity. Currently, utilization of both mineral and organic fertilizers have been accelerated as a move to increase soil productivity. Agricultural residues from oil palm, rubber and cocoa plantations are also applied to land as organic fertilizers, along with mineral fertilizers.

The palm oil mill effluent (POME) contributes a large portion of the agricultural waste. This waste if not utilised correctly can cause environmental pollution. Application of POME to soil has been shown to increase the growth of oil palms and other crops (Lim et al., 1984). Land application of POME has also been shown to improve soil physical, chemical (Lim, 1985) and biological properties (Radziah, 1994).

Application of high rates of raw POME has been proven to inhibit the growth of several plant species. This effect could probably be due to the unfavourable physical, chemical or biological properties of POME or the unfavourable soil properties that arise as a result of POME application. Earlier studies with oil palm seedlings indicated that the presence of high lipid and volatile substances in POME could cause clogging of soil, which subsequently will inhibit growth of plant roots (Mohd. Nazeeb et al., 1984). Zulkifli and Rosmin (1990) observed a reduction in growth of vegetables grown on soil applied with undecomposed POME. In view of this, POME is normally left to decompose in the soil for 4 to 6 weeks before it is utilised as an organic fertilizer or soil conditioner.

Decomposition of POME has been proven to be essential in reducing its inhibitory effects on plant growth. Decomposition process also allows release of nutrients such as N and P to the growing plants (Mohd. Hashim,1990; Palaniappan et al., 1984). Microorganisms are known to be responsible in decomposing POME in soil. Palaniappan et al. (1984) had earlier shown the presence of several fungal species at different stages of POME decomposition. These microorganisms could probably be responsible in breaking down the plant inhibitory compounds present in POME.

The inhibitory effect of raw POME on plant growth is similar to the phytotoxicity exhibited by other types of crop residues. Most crop residues such as rice, wheat, corn, rye, oats, straws, grasses and legumes contain water-soluble substances which inhibit germination and growth of a number of seedlings. (Rice, 1984). Inhibition to plant growth has been closely associated with the presence of phenolic substances. High amounts of toxic compounds are usually leached out from the organic residues into soils. Toxic compounds are also produced by the microorganisms during residue decomposition. Several phenolic acids mainly ferulic, *p*-coumaric, syringic, vanillic and *p*-hydroxybenzoic have been quantitatively isolated and estimated in plant residues.

The degree of phytotoxicity depends on the types of organic residue, decomposition period and the sensivity of plant root system (Zucconi and de Bertoldi, 1984). Phytotoxicity from plant residues was maximum at the early stage of decomposition. The amount however diminished with prolonged decomposition period (Guenzi et al., 1967). Toxic effect from wheat and oat residues disappeared after 8 weeks of field exposure, while corn residues remained toxic even after 8 weeks of decomposition (Kimber, 1973).

Several microorganisms are capable of utilising phenolic acids as their source of carbon and energy. Some of the bacterial and fungal species are *Pseudomonas* sp., *Syncephalastrum racemosus*, *Cephalosporium curtipes, Rhodotorula rubra* and *Penicillium* sp. (Henderson, 1956; Turner and Rice, 1975). The ability of these microorganisms in reducing the phytotoxicity of these phenolic compounds has not been widely studied. Sparling and Vaughan (1981) however showed that inoculation of wheat seedlings with *Pseudomonas* and *Syncephalastrum racemosus* reduced the toxicity of the phenolic acid present in the growth medium.

A series of studies were undertaken to test the hypothesis that one type of POME known as palm oil meal (POMeal), may contain water-soluble organic compounds which could inhibit growth of other plants. Inoculation of POMeal with microorganisms isolated from either soil or decomposed POMeal could probably reduce these inhibitory effects. If this holds true, utilization of POMeal as an environment friendly organic fertilizer for crop production offers a bright potential. Hence, laboratory and glasshouse experiments were conducted to :-

i) determine the inhibitory effect of different levels of raw POMeal on growth of vegetables (Experiment 1),

ii) determine the effect of decomposition on phytotoxicity levels of raw POMeal (Experiment 2),

iii) extract and identify the water-soluble phytotoxic compounds present in raw POMeal (Experiment 3),

iv) study the degradation of *p*-coumaric and vanillic acids by *Pseudomonas* sp. and *Penicillium* sp. (Experiment 4) and,

v) determine the effect of decomposition period and microbial inoculation in reducing the POMeal phytotoxicity, and hence its effect on growth of spinach (Experiment 5).

Experiments 2, 3 and 4 were carried out in the laboratory, while Experiments 1 and 5 were conducted in the glasshouse.

CHAPTER TWO

LITERATURE REVIEW

Utilization of Organic Inputs in Malaysia

Soils in the tropic are usually infertile as a result of intense and prolonged weathering. In Malaysia, the organic material in the top soil is rapidly being decomposed and the soils are generally heavily leached, acidic and low in exchangeable bases, percentage base saturation and cation exchange capacity (CEC) (Law and Tan, 1977). These, together with the overall mismanagement of land lead to the rapid decline in soil productivity. Excessive use of chemical fertilizers could also bring adverse effects on the environment. Proper management of both mineral fertilizers and organic inputs to improve and sustain soil fertility is hence crucial.

Currently, utilization of organic materials in the agriculture sector is increasing rapidly. Agricultural residues from oil palm, rubber and cocoa plantations are being recycled and applied to land as organic fertilizers along with mineral fertilizers, in attempts to increase crop yield and reduce cost of mineral fertilizers. Vegetable and fruit cultivation constitutes the largest user of organic fertilizers. The application of chicken dung together with mineral fertilizers has been shown to increase vegetable production compared to mineral fertilizer alone. In the Cameron

Highlands, it was common to apply 5-10 mt ha^{-1} chicken dung for each new crop (Vimala and Chan, 1990). Sometimes as high as 20 mt ha^{-1} chicken dung was applied to newly opened areas. However, application of this material has been reduced when the soil became saturated with organic colloid. The use of organic input is even more crucial for crops grown on sandy tailing and Bris soils which are low in nutrients and CEC, and low water holding capacity (Lim et al., 1981).

Chicken dung has been the main source of organic input applied to several fruit growing areas of Peninsular Malaysia (Aini et al., 1992). About 5-10 kg is applied into the planting hole with subsequent addition during the growing stage. However, application of chicken dung into soil must be done with caution, as continuous and excessive use of chicken dung has recently been shown to be detrimental to the soil flora and fauna (Azizah, pers. comm., 1995). A recent shift in fruit cultivation in sandy soil is towards the utilization of oil palm empty fruit bunches and palm oil mill effluent (POME) as soil amendments (Lim et al., 1991). Research in the recycling of some other agricultural wastes such as rice straw and stubbles, green manures and POME, incorporated with mineral fertilisers for use on various crops is being conducted.

The Advantages of Organic Inputs and Soil Organic Matter

The terms `organic input' and `soil organic matter' have recently been used instead of organic matter, to better evaluate the management and effect of the organic material on nutrient cycling and sustainable soil productivity (Sanchez et al., 1989).

Organic input refers to the above and below ground litter, crop residues, mulches, green and animal manures. These constitute the external source of nutrients and organic carbon. Soil organic matter (SOM) is the result of partial or complete transformation of these organic inputs in soil and is located below the soil surface. In general, application of organic input improves soil physical, chemical and biological properties which will positively affect nutrient cycling and plant productivity. The functions of the organic inputs are as discussed below.

Physical Functions

The transformation of the fibrous portion of organic materials is responsible for the formation of water stable aggregates. This will subsequently improve soil permeability and aeration. The high absorbing power of the material also stimulates granulation of sandy soils and improves its nutrient and water holding capacity. It also affects the infiltration rates, total quantity of water in the soil as well as evaporation from the soil surface (Tate, 1987). Mulching also helps reduce the soil temperature to a level favourable for plant growth. The improvement in soil structure subsequently stimulates root growth for better absorption of nutrients and water.

Chemical Functions

The decomposition and mineralization processes enable the organic materials to release nutrients such as nitrogen, phosphorus, and sulphur for plant growth. The rate of nutrient release depends on the rate of decomposition, which in turn is affected by temperature, moisture, soil texture, mineralogy, as well as the quantity, quality, placement and application time of the organic inputs (Fernandes and Sanchez, 1990). In natural system, sustainable productivity relies on the efficient and rapid decomposition of organic inputs (Swift et al., 1979). Since mineralization is dependent on the composition of the organic material, the release of N and P therefore occurs only when N and P-rich organic inputs are used.

The cation exchange capacity of soils increases with addition of the organic materials. Negative charges created by the dissociation of the carboxyl and hydroxyl groups of the organic material, enhances the soil to retain nutrients from the mineralization process or from the applied fertilizers, while increasing the buffering capacity of the soil. The humus fraction in SOM contributes to the mineral balance in soil and also accounts for the chelation of some micronutrients such as iron and zinc and their absorption by plant (Zucconi and de Bertoldi, 1987).

Biological Functions

Carbon in organic inputs is the main source of energy for the soil microorganisms. Addition of organic materials into the soil increases the activities of several species of bacteria, fungi and actinomycetes (Alexander, 1977; Paul and Clark, 1989). These microorganisms are involved in the transformation of the organic inputs to humus. The rate of microbial decomposition depends on: the chemical composition of the organic materials applied such as the carbohydrates, the C/N ratio and the quantity of lignin present. Addition of materials with high C/N ratio