

**Research Article**

**Potential use of soil-born fungi isolated from treated soil in Indonesia to degrade glyphosate herbicide**

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**Abstract:** The glyphosate herbicide is the most common herbicides used in palm-oil plantations and other agricultural in Indonesia. In 2020, Indonesian government to plan the development of oil palm plantations has reached 20 million hectares of which now have reached 6 million hectares. It means that a huge chemicals particularly glyphosate has been poured into the ground and continues to pollute the soil. However, there is no report regarding biodegradation of glyphosate-contaminated soils using fungal strain especially in Indonesia. This study was to observe the usage of Round Up as selection agent for isolation of soil-born fungi capable to grow on glyphosate as a sole source of phosphorus. Five fungal strains were able to grow consistently in the presence of glyphosate as the sole phosphorus source and identified as *Aspergillus* sp. strain KRP1, *Fusarium* sp. strain KRP2, *Verticillium* sp. strain KRP3, *Acremonium* sp. strain GRP1 and *Scopulariopsis* sp. strain GRP2. This indicates as their capability to utilize and degrade this herbicide. We also used standard medium as control and get seventeen fungal strains. The seventeen fungal strains were identified as species of *Botrytis*, *Fusarium*, *Aspergillus*, *Penicillium*, *Verticillium*, *Trichoderma* and *Paecilomyces*. These results show the reduction in the number of fungal strains on solid medium containing glyphosate. Of the five isolated fungal species, *Verticillium* sp. strain KRP3 and *Scopulariopsis* sp. strain GRP2 were selected for further study based on their highest ratio of growth diameter. This study indicates that treatment of soil with glyphosate degrading fungus would be useful in some areas where this herbicide is extensively used.

**Keywords:** biodegradation, glyphosate, *Scopulariopsis*, *Verticillium*.

**Introduction**

Glyphosate was introduced in 1974 for non-selective weed control and sales took off in the late 1990s, after Monsanto created its brand of Roundup, frequently sold under the brand name Roundup. Roundup is the most common herbicides used in palm-oil plantations and other agricultural. Indonesia is the second producer of palm oil after Malaysia, together with a total production of 80% of the total world production of palm oil. In 2020, Indonesian government to plan the development of oil palm plantations has reached 20 million hectares of which now have reached 6 million hectares. It means that a huge chemicals particularly glyphosate has been poured into the ground and continues to pollute the soil.

The excessive use and large scale synthesis of glyphosate cause a number of environmental problems as reported by Buffin and Jewell (2001). Glyphosate is toxic to soil organisms and beneficial arthropod predators. Penaloza-Vazquez et al. (1995) reported that glyphosate remains unchanged in the soil for varying lengths of time, because of its adsorption on clay particles and organic matter present in the soil. This condition makes this herbicide very persistent in soils and sediments. Factually, glyphosate herbicide has met a success commercially as an effective herbicide. This success, also supported by the statement as environmentally friendly, has encouraged extensive studies on its biodegradation by soil microorganisms.

Glyphosate's primary route of decomposition in the environment is through microbial degradation. The herbicide is inactivated and biodegraded by soil microbes at rates of degradation related to microbial activity in the soil and factors that affect this activity (Eriksson, 1975). The biological degradation process is carried out under both aerobic and anaerobic conditions by soil microorganisms. Rates of decomposition depend on soil and microbial population types.

Studies of glyphosate degrading microbia have involved selection and isolation of pure microbial culture strains with enhanced or novel detoxification capabilities for potential uses in biodegradation of polluted soil and water. The available reference on the microbial degradation of glyphosate is wide spread among bacteria. However, the study on biodegradation of glyphosate by fungal species is lacking. Only the degradation process by *Penicillium* (Bujacz et al., 1995; Pothuluri et al., 1998; Klimek et al., 2001) and *Fusarium* (Sudol and Krzyśko-Lupicka, 2005) have been extensively characterized. Interestingly, all of the above isolates were able to utilize glyphosate as sole source of phosphorus.

In this study we report the use of glyphosate herbicide as screening agent of the sole phosphorus source for isolation of soil-born fungi from treated soil in Indonesia. We also observed the growth response of the fungi in broth medium containing glyphosate as their characteristics of glyphosate degradation process.

Isolation of fungal strains had been previously obtained from other soil samples which had been previously exposed by glyphosate (Krzyśko-Lupicka and Orlik, 1997) and also from industrial activated sludge (Hallas et al., 1988). However, there is no report regarding to soil-born fungi from treated soil in Indonesia.

## Materials and Methods

### Soil

Soil samples were obtained from two areas of fruit and vegetable plantations at Malang, East Java, Indonesia. This area has been exposed by glyphosate for more than 10 years with twice a year in application. They were collected from depths of 0-15 cm from four sites, placed in sterile polyethylene bag and mixed well. All samples were transported immediately to the laboratory and stored at 4°C in refrigerator.

### Chemicals

Glyphosate known as Roundup® (containing 480 g active ingredient/L of glyphosate, Nissan

Chemical) was purchased from a local store in Ube, Yamaguchi, Japan. All other chemicals were of the highest purity commercially available.

### Isolation of fungal strains

Soil samples were processed in an isolation process using direct inoculation of screened immersion plate, containing standard Rose Bengal Agar C (RBAC) which consisted of 5 g of soy peptone, 10 g of glucose, 1 g of  $\text{KH}_2\text{PO}_4$ , 0.5 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.05 g of Rose Bengal, 15 g of agar and 0.1 g of chloramphenicol at pH 5.7. Modified RBAC was prepared same as description on standard RBAC however  $\text{KH}_2\text{PO}_4$  was replaced by glyphosate herbicide (RoundUp) on equivalent concentration to the field application rate (7.2 mg/ml). The plates were incubated for 5 days at 25°C. Individual strains were isolated based on the distinct of the colony morphology. Young active of fungal mycelia from each strain was purified using single spore technique. The above steps were repeated by re-isolation of fungal mycelia on modified Rose Bengal Agar C. The selected fungal strains that consistently grew on modified RBAC were re-streaked on Potato Dextrose Agar (PDA).

### Taxonomic Identification

Individual strains isolated from modified RBAC were then cultivated on Malt Extract Agar, PDA and Czapek Agar for their taxonomic investigation based on their morphology of colony and cell. Identifications were conducted using identification keys and methods following Domsch et al. (1993).

### Ratio of growth diameter

Ratio of growth diameter was observed to find out two strains which have the highest capability of growth on modified RBAC containing glyphosate as the sole source of phosphorus as described in "isolation of fungal strains". The ratio of growth diameter was determined based on colony diameter on PDA as control plate. The measurement of mycelial growth was performed according to the method described by Shim et al. (2005). A 5 mm diameter plug of 5 days old cultures of fungal strain from PDA culture was cut with cork borer and then placed in the center of each agar plate at pH 6, incubated for 7 days at 27°C in three replicate.

The ratio of growth diameter =  $[M/S]$ , where M is growth diameter on treatment (modified RBAC), and S is growth diameter on control (PDA)

### Growth profile

The observation of growth profile was examined in 250 ml Erlenmeyer flask containing 100 ml Czapek broth medium, which was inoculated with spore suspension ( $2 \times 10^7$  spores/mL), and pH of the medium was maintained at 5.6-6. Each liter of Czapek broth medium consisted of 30 g of sucrose, 3 g of  $\text{NaNO}_3$ , 1g of  $\text{K}_2\text{HPO}_4$ , 0.25 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g of KCl and 10 mg of  $\text{FeSO}_4$ . The same medium was removed in  $\text{K}_2\text{HPO}_4$  as the absence of phosphate medium (control). Thus, when glyphosate was used as sole source of phosphorus,  $\text{K}_2\text{HPO}_4$  was replaced by glyphosate at a final concentration of 10 mM (Krzyśko-Lupicka et al., 1997). All glassware were washed with 1 N HCl and rinsed with deionized water to remove contaminating phosphate before use and then glyphosate herbicide was added after medium sterilization. Every four days the cultures were filtered and dry mass of mycelium was determined. The average values were obtained from three replicates.

### Results and Discussion

#### Isolation soil-born fungi

The results of isolation of soil-born fungi from treated soil show only five fungal strains were

able to grow consistently on modified RBA-C, however on standard medium resulted seventeen fungal strains (Table 1). These results show the reduction in the number of fungal strains on solid medium containing glyphosate. Five fungal strains isolated from solid medium containing glyphosate were then identified as *Aspergillus* sp. strain KRP1, *Fusarium* sp. strain KRP2, *Verticillium* sp. strain KRP3, *Acremonium* sp. strain GRP1 and *Scopulariopsis* sp. strain GRP2. Seventeen fungal strains isolated from standard medium were identified as species of *Botrytis*, *Fusarium*, *Aspergillus*, *Penicillium*, *Verticillium*, *Trichoderma* and *Paecilomyces*. The previous studies (Krzyśko-Lupicka and Orlik, 1997) reported that *Mucor*, *Trichoderma* and *Fusarium* were obtained in media containing glyphosate whereas *Penicillium*, *Cladosporium*, *Sclerotinia* and *Scopulariopsis* dominated in standard medium. The reduction of total number of fungal strains in this experiment corresponds to Busse et al. (2001) who reported that culture able bacteria and fungi are usually diminished in number when extracted from soil and then cultivated on solid medium containing glyphosate. Glyphosate herbicide contained in medium is expected to be toxic to the fungi and probably disable the organism to synthesize the needed aromatic amino acids

Table 1. Fungal strains isolated on standard and modified RBAC with glyphosate herbicide as screening agent of the sole source of phosphorus.

| Standard Rose Bengal Agar C        | Modified Rose Bengal Agar C    |
|------------------------------------|--------------------------------|
| Number of strains: 17              | Number of strains: 5           |
| <i>Botrytis</i> spp. 2 strains     | <i>Aspergillus</i> sp. KRP1    |
| <i>Fusarium</i> spp. 5 strains     | <i>Fusarium</i> sp. KRP2       |
| <i>Aspergillus</i> spp. 3 strains  | <i>Verticillium</i> sp. KRP3   |
| <i>Trichoderma</i> spp. 2 strains  | <i>Acremonium</i> sp. GRP1     |
| <i>Penicillium</i> spp. 2 strains  | <i>Scopulariopsis</i> sp. GRP2 |
| <i>Verticillium</i> spp. 2 strains |                                |
| <i>Paecilomyces</i> ssp. 1 strain  |                                |

#### Ratio of Growth diameter

Figure 1 shows the ratio of growth diameter of five fungal strains on modified RBAC containing glyphosate as the sole source of phosphorus which was determined based on growth diameter on PDA as control plate. *Scopulariopsis* sp. strain GRP2 has the highest ratio of growth diameter, then following by *Verticillium* sp. KRP3, *Acremonium* sp. GRP1, *Aspergillus* sp. KRP1, and the lowest is *Fusarium* sp. KRP2.

#### Growth profile

The growth profile of *Verticillium* sp. strain KRP3 and *Scopulariopsis* ssp. strain GRP2 were further monitored by their mycelial dry mass for 24 days, using Czapek broth medium in full (complex medium), Czapek broth medium containing glyphosate as sole phosphorus source and Czapek broth medium in the absence of any phosphorus source (control).

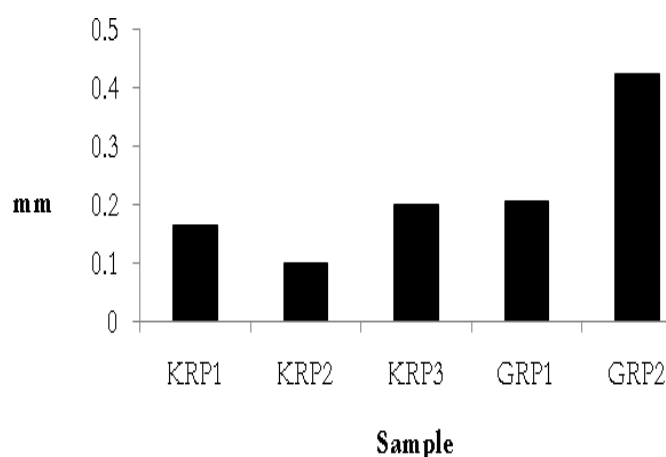


Figure 1. Ratio of growth diameter of five fungal strains on modified Rose Bengal Agar C containing glyphosate as the sole source of phosphorus which was determined based on growth diameter on PDA as control plate

Figures 2 and 3 show both of fungal species have similar pattern in their growth profile on three kinds of medium and showed appreciable growth in the culture medium containing glyphosate as sole phosphorus source. Their growth profile in full medium consistently increased till the end of the monitoring (24 days of incubation). In the medium containing glyphosate as sole phosphorus

source, the exponential phase was during 4 days and there was a progressive increase during 12 days of incubation; a lag phase during 12 to 20 days of incubation and then increased again after 20 days. Growth profile of both fungi in control medium was very low and a lag phase after 8 days till the end of monitoring.

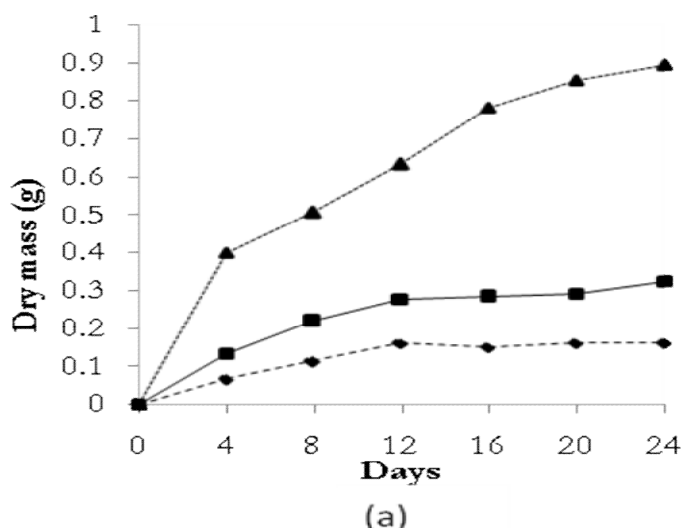


Figure 2. Growth profile of *Scopulariopsis* sp. strain GRP2 on full Czapek medium (▲), Czapek medium containing glyphosate as the sole source of phosphorus (◆) and Czapek medium in the absence of any phosphorus source (■).

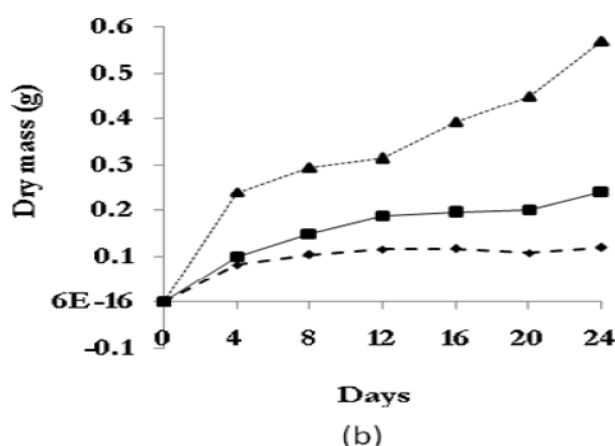


Figure 3. Growth profile *Verticillium* sp. strain KRP3 on full Czapek medium (▲), Czapek medium containing glyphosate as the sole source of phosphorus (◆) and Czapek medium in the absence of any phosphorus source (■).

## Conclusions

Only five fungal strains were able to grow consistently in the presence of glyphosate as the sole phosphorus source and identified as *Aspergillus* sp. strain KRP1, *Fusarium* sp. strain KRP2, *Verticillium* sp. strain KRP3, *Acremonium* sp. strain GRP1 and *Scopulariopsis* sp. strain GRP2. Amongst the five fungal species screened, *Verticillium* sp. strain KRP3 and *Scopulariopsis* sp. strain GRP2 showed the highest ratio of growth diameter. The growth profile of *Verticillium* sp. strain KRP3 and *Scopulariopsis* sp. strain GRP2 showed appreciable growth in the culture medium containing glyphosate as sole phosphorus source and has similar pattern in the existence of a lag phase. This study indicates that treatment of soil with glyphosate degrading fungus would be useful in some areas where this herbicide is extensively used.

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