The effectiveness of bioherbicide made from nutsedge tuber powder (*Cyperus rotundus* L.) in various dosage and application time

A I Sulistiani¹, M A Chozin²*, D Guntoro² and Suwarto²

*Email: ma_chozin@yahoo.com

**Abstract.** Nutsedge (*Cyperus rotundus* L.) is a very adaptive weed, therefore it is very difficult to be controlled. Besides, the *Cyperus rotundus* has allelopathic compounds that can interfere with the growth of the main plant. The allelopathic compound has the potential to be used as a pre-emergence bioherbicide for controlling broadleaf weeds and grass. The aim of this study was to determine the effectiveness of bioherbicides made from nutsedge tuber powder sources at various dosage and application times. This research was conducted from August to October 2019, used CRD with three replications. The first factor was the dosage (D); 45.00 and 67.50 kg powder ha⁻¹. The second factor was the application of time (T); 2 DBP, 0, 2, 4 DAP so that there were 8 treatment units. The result showed that the application of bioherbicides made from nutsedge tuber powder with pellet formulations on 0 to 4 DAP was effective in inhibiting the germination and growth of all test weeds, the best application time varies between test weeds. In general, a dosage of 67.50 kg tuber powder ha⁻¹ was more effective in inhibiting germination and growth of weed sprouts as compared to a dosage of 45.00 kg tuber powder ha⁻¹.

1. **Introduction**

Weeds are unexpected growing plants. They grow wild and on cultivated land. The weeds growing around cultivated plants can cause various problems, such as competition for nutrients, light, water, as well as host for some pathogens so that they can reduce production yields of cultivated plants. Weeds are one of the biotic factors that can inhibit the growth and production of cultivated plants. Many weed species cause losses in crop cultivation, as a consequence the number and quality of yields will be reduced [1].

Recently, research on the use of the natural resource as an alternative to synthetic herbicides is being developed. Plants that contain allelopathic compounds are known to be used as substitutes for synthetic herbicide, as a bioherbicide source that environmentally friendly and easy to obtain. Nutsedge (*Cyperus rotundus* L.) is an important weed in the horticultural field. Several previous studies have been carried out on nutsedge that is potential as a biological herbicide (bioherbicide).

*C. rotundus* L. was reported to be effective in inhibiting the germination of broadleaf weed seeds and has the potential to be developed as a pre-emergence bioherbicide [2]. Nutsedge extract contains more secondary metabolites than in the plant crown [3]. It can inhibit the growth of plumules and radicles in *Borreria alata* [4]. Nutsedge extracts can also control *Richardia brasiliensis* weeds in soybean crops [5]. Chairannisa and Chozin [6] also reported that the higher the concentration of nutsedge extract, the more it suppressed the germination of *Asystasia gangetica* on the paper. Recent studies have reported that Nutsedge extract show the highest content of α cyperone (sesquiterpene) is obtained from the whole tuber extract. These compounds are effective in inhibiting the germination of lettuce [7].

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The herbicide application aims to control weeds without damaging the main crop. Herbicide selectivity often becomes the problem. Selectivity is determined by the dosage or concentration of the herbicide solution. Apart from selectivity, another factor that affects the effectiveness of herbicide application is the application time. The timing of the herbicide application is related to the type of weed target. The aim of this study was to determine the effectiveness of dosage and application time of bioherbicides made from pellet formulation of Nutsedge extract in inhibiting the germination of weed seeds at various doses and application times.

2. Materials and methods

The research was conducted from August to October 2019. The research was conducted at 1) Post-Harvest Laboratory, Biomass, and Spectrophotometry, Faculty of Agriculture, IPB for making tuber powder extract. 2) Cikabayan Experimental Station, Faculty of Agriculture, IPB for field testing of the bioherbicides. The tools used were laboratory equipment for extraction, sample bottles for storing weed seeds, 40 × 30 × 5 (cm) tray, blender, oven, sieve (5 mesh), tweezers, scissors, analytical balance, camera, ruler, stationery, and other standard laboratory equipment.

The materials used were tuber powder (C. rotundus L.), test plants (Asystasia gangetica, Amaranthus gangeticus, Lettuce sativa, Echinochloa crusgalli, and Sorghum bicolor), aquades, and soil. The seeds used were confirmed to be in physiological ripe conditions with black characteristics and not in a state of dormancy. Weed seeds to be firstly sown and sterilized to prevent fungal attacks by soaking them in 1% NaClO for 10 minutes and rinsing them with distilled water three times to remove the remaining NaClO residue on the seeds. The soil was sterilized before, so the weed seeds in the soil died. Sterilization used the steaming method for 8 hours.

This study used a two-factor completely randomized design (CRD). The following is the additive linear model of the two-factor RAL trial design, as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$  \hspace{1cm} (1)

$Y_{ijk}$ = The result of observations on the dose factor level-$i$, time factor level-$j$, and repetition level-$k$

$\mu$, $\alpha_i$, dan $\beta_j$ = Mean, the main influence of the dose factor and the main influence of the time factor

$(\alpha\beta)_{ij}$ = Interaction of formulation factors and dosage factors

$\epsilon_{ijk}$ = Normal diffuse random effects

The first factor was the dosage of the nutsedge extract (D) which consisted of 2 levels (45.00 kg and 67.50 kg powder ha$^{-1}$). The second factor was the time (T) of the application consisting of four levels, namely 2 days before planting (DBP), 0 days after planting (DAP), 2, 4 days after planting (DAP), so that there were 8 treatment units.

The pellet formulation is obtained from nutsedge tuber powder mixed with corn-powder in a ratio of 1:10, adding cooking oil as an adhesive, then printed using a pellet machine.

The bioherbicide formulation made from nutsedge tuber powder is made in the form of pellets (granules). The steps to make pellets i.e: a) nutsedge tuber powder. The nutsedge extract is cleaned, washed, then cleaned from the roots and immediately dried using an oven at 60 °C for 3 days, then the nutsedge extract is blended until becoming powder and sieved with a 5 mesh sieve, then the nutsedge tuber powder is ready to use. b) granules/pellets; The making process of pellets from nutsedge tuber powder was carried out at the Feed Industry Laboratory of IPB, Bogor. Making pellets using a pellet machine with a ratio of ingredients and carriers (corn powder), with ratio 1:10. The finished pellets are ready to be applied.

The experiment was carried out using 50 test weeds were planted in a tray filled with sterile soil. Bioherbicide made from nutsedge tuber powder was applied according to the treatment level. The application was spread evenly over the soil surface in a tray of weed seed nurseries. Water application was only used to calculate the relative effectiveness of the treatment on germination parameters. It was
not used in the analysis of diversity. Effective treatment can suppress the growth of weeds by more than 50% compared to water application.

Observation of the percentage of germination (PP) at 14 days after planting (DAP) was carried out every day, based on the following measurement methods:

\[
PP \text{ 14 DAP} = \frac{\text{the number of seeds germinated}}{\text{Total number of seeds tested}} \times 100
\]  

Measurement of germination plumules and radicles. Destructive measurements are made of the growth sprouts by measuring the plumules and radicles; the radicle is measured from the tip of the growing point to the base of the root, and the radicle from the base of the root to the tip of the root. The time to take sprouts was at the age of 7 and 14 DAP by taking 3 samples/tray.

Observation of normal and abnormal Sprouts. The time to take sprouts was at the age of 7 and 14 DAT.

The growth rate was observed every day, the growth rate can be calculated using the following formula:

\[
K_{ct} = \sum_{i=0}^{t} \frac{\%SN}{Etma1}
\]  

Note:
- \(K_{ct}\): Speed growing
- \(\%SN\): Percent normal sprouts
- \(Etma1\): Observation of seedling growth every 24 hours

The analysis of quantitative data is obtained from the experimental results. If the treatment shows effects, then a further test will be carried out using the Duncan Multiple Range Test (DMRT) at the 5% level.

3. Results and discussion

3.1. Germination percentage

Application of bioherbicide made from nutsedge powder at the dosage level affected the percentage of final germination of \(L.\ sativa\) and \(S.\ bicolor\) seeds but did not affect \(A.\ gangetica, A.\ gangeticus\) and \(E.\ crusgalli\). Meanwhile, the time of application of bioherbicide made from nutsedge powder affected the percentage of final germination of \(S.\ bicolor\) seeds but did not affect the other four types of weeds.

Table 1. The average percentage of seed germination of \(A.\ gangetica, A.\ gangeticus, L.\ sativa, E.\ crusgalli,\) and \(S.\ bicolor\) at various dosage and times of application of bioherbicide made from nutsedge powder

| Treatment | Germination of 14 DAP (%) | \(A.\ gangetica\) | \(A.\ gangeticus\) | \(L.\ sativa\) | \(E.\ crusgalli\) | \(S.\ bicolor\) |
|-----------|---------------------------|-------------------|-------------------|----------------|-----------------|----------------|
| Dosage (Kg ha\(^{-1}\)) | | | | | | |
| 45.00     | 46.33 (36.82)             | 35.83 (44.02)     | 77.67 a (17.96)   | 9.14 (83.48)   | 27.00 a (43.75) |
| 67.50     | 43.33 (40.91)             | 37.17 (41.92)     | 56.00 b (40.85)   | 8.83 (84.04)   | 19.67 b (59.02) |
| Time      |                           |                   |                   |                |                  |                |
| 2 DBP     | 46.67 (36.36)             | 37.00 (42.19)     | 63.33 (33.10)     | 9.36 (83.08)   | 18.50 b (61.46) |
| 0 DAP     | 42.33 (42.27)             | 37.00 (42.19)     | 58.67 (38.03)     | 9.19 (83.39)   | 18.00 b (62.50) |
| 2 DAP     | 36.33 (50.46)             | 36.33 (43.23)     | 73.00 (22.89)     | 8.95 (83.82)   | 24.50 b (48.96) |
| 4 DAP     | 54.00 (26.36)             | 35.67 (44.27)     | 72.33 (23.60)     | 8.43 (84.76)   | 36.00 a (25.00) |
| Water     | 73.33                     | 64.00             | 94.67             | 55.33          | 48.00           |

Note: Based on the DMRT at the 5% level, the numbers accompanied by different letters in the same column show significantly different. DBP; the day before planting, DAP; days after planting, the numbers in brackets indicate inhibited
Table 1 shows that the dosage and application time of bioherbicide made from nutsedge extract powder compared to the control was effective in reducing the germination percentage of *A. gangetica*, *A. gangeticus*, *L. sativa*, *E. crusgalli*, and *S. bicolor* seeds. The average germination percentage of *A. gangetica* seeds is (36.33 - 54.00%), *A. gangeticus* is (35.67 - 37.17%), *L. sativa* is (56.00 - 77.67%), *E. crusgalli* is (8.43 - 9.36%), and *S. bicolor* is (18.00 - 36.00%).

In general, the best application dosage was 67.50 kg of powder ha$^{-1}$ in *A. gangetica*, *L. sativa*, *E. crusgalli*, and *S. bicolor*, except for *A. gangeticus*. The dosage of 67.50 kg of powder ha$^{-1}$ is effective in suppressing the germination percentage compared to a dosage of 45 kg of powder ha$^{-1}$, this strengthened the results of previous studies conducted by Delsi [8], Andhini et al. [9], Kusuma et al. [4], Chairannisa and Chozin [6], and Nuryana et al. [7] the higher concentration of nutsedge extract applied, the higher the level of inhibition.

The application time of bioherbicide made from nutsedge powder showed the ability to suppress different germination for each type of weed: *A. gangetica* (26.36 - 50.46%), *A. gangeticus* (41.92 - 43.23%) *L. sativa* (17.96 - 77.67%), and *E. crusgalli* (83.08 - 84.76%), and *S. bicolor* (25.00 - 62.50%). Lesilolo et al. [10] reported that the higher the number of days required for a germination process, the lower the index value of the germination speed. This shows that each weed has a different response. The difference is assumed influenced by the characteristics of each weed seed that is different from one another. Alghofar et al. [11] reported that sengon seeds have a dormancy period caused by the hard texture of the seed coat, which inhibits the water and oxygen absorption into the seeds.

### 3.2. Length of plumules and radicles

The result showed that the interaction between dose level and application time of bioherbicide made from nutsedge powder affected the length of *E. crusgalli* and *L. sativa* plumules, as well as the length of *L. sativa* radicles. The interaction effect between dosage level and application time of bioherbicide made from tuber powder is shown in Table 2.

**Table 2.** Effect of dosage and time interactions of bioherbicide application made from nutsedge powder on plumule length 7 DAP in *E. crusgalli* and *L. sativa*

| Dosage tuber powder | Application Time | Plumule length *E. crusgalli* (cm) | Plumule length *L. sativa* (cm) | Radicles length *L. sativa* (cm) |
|---------------------|------------------|-----------------------------------|---------------------------------|---------------------------------|
|                     | 2 DBP 0 DAP 2 DAP 4 DAP |                                 |                                 |                                 |
| 45.00 kg ha$^{-1}$  | 2.72 ab 1.50 d 2.36 abc 2.45 abc |                                 |                                 |                                 |
| 67.5 kg ha$^{-1}$   | 1.88 d 3.05 a 2.20 bc 2.25 bc |                                 |                                 |                                 |
| Water               | 3.33 |                                 |                                 |                                 |
| 45.00 kg ha$^{-1}$  | 0.99 a 1.04 a 0.70 b 0.75 b |                                 |                                 |                                 |
| 67.5 kg ha$^{-1}$   | 1.03 a 0.78 b 0.80 b 0.76 b |                                 |                                 |                                 |
| Water               | 1.20 |                                 |                                 |                                 |

Note: Based on the DMRT at the 5% level, the numbers accompanied by different letters in the same column showed significantly different. DBP; the day before planting, DAP; days after planting

Table 2 shows that all combinations of dosage and application time of bioherbicides made from nutsedge powder were effective in inhibiting plumule length in *E. crusgalli* and length of *L. sativa* plumules and radicles. The average length of *E. crusgalli* plumules ranged from 1.50 - 3.05 cm, lower than the control 3.33 cm, the mean length of *L. sativa* plumules, and radicles (0.70 - 1.04 cm and 1.37 - 1.96 cm) were lower than that of controls 1.20 and 3.00 cm.
The application of bioherbicides in various test plants gave an effect of suppression on the length of *E. crusgalli* and *L. sativa* plumules, as well as the length of *L. sativa* radicles. This showed that the application time of bioherbicide made nutsedge powder on accession 0 days after planting to 4 days after planting was effective in inhibiting the length of the tested plumules and radicles of weeds. This information indicates that bioherbicides made from nutsedge powder have the potential to be bioherbicide for pre-emergence and early post-emergence. The result reinforced the results from previous research, that the application of nutsedge powder from various accessions has the ability to suppress the growth of broadleaf weeds in soybean fields up to 2 WAP [5]. Nutsedge powder inhibits the growth of *B. alata* plumules and radicles at 2 days after seeding [4]. Plant height and root length are determined by the length of the plumules and radicles.

### 3.3. Abnormal sprouts

The results showed that the dosage of bioherbicide made from nutsedge powder on the tested weeds had a different effect on abnormal sprouts on *A. gangetica* and *S. bicolor*

**Table 3.** The average percentage of abnormal sprouts of *A. gangetica, A. gangeticus, L. sativa, E. crusgalli*, and *S. bicolor* at various doses and times of application of bioherbicide made from nutsedge powder

| Treatment | A. gangetica | A. gangeticus | L. sativa | E. crusgalli | S. bicolor |
|-----------|--------------|---------------|-----------|--------------|-----------|
| **Dosage (Kg ha⁻¹)** |              |               |           |              |           |
| 45.00     | 16.00        | 6.50          | 17.5      | 16.50        | 3.17 b    |
| 67.50     | 14.83        | 8.00          | 17.5      | 14.50        | 5.50 a    |
| **Time**  |              |               |           |              |           |
| 2 DBP     | 17.00        | 5.67 b        | 18.00     | 17.00        | 4.00 ab   |
| 0 DAP     | 12.00        | 10.00 a       | 15.33     | 16.33        | 3.00 b    |
| 2 DAP     | 13.00        | 7.33 ab       | 14.00     | 14.00        | 4.00 ab   |
| 4 DAP     | 19.67        | 6.00 b        | 22.67     | 14.67        | 6.33 a    |
| **Water** | 10.00        | 1.33          | 10.00     | 13.33        | 0.67      |

Note: Based on the DMRT at the 5% level, the numbers accompanied by different letters in the same column show significantly different. DBP; the day before planting, DAP; days after planting

The highest percentage of abnormal sprouts was in the tested weeds *A. gangetica* and *E. crusgalli* at a dosage of 45.00 kg of ha⁻¹ powder, while in *A. gangeticus* and *S. bicolor* the highest percentage of abnormal sprouts was at a dosage of 67.50 kg powder ha⁻¹. Table 3 shows the average percentage of normal and abnormal sprouts of *A. gangetica* (12.00 - 19.67%), *A. gangeticus* (5.67 - 10.00%), *L. sativa* (15.33 - 22.67%), *E. crusgalli* (14.00 - 17.00%), and *S. bicolor* (3.00 - 6.33%). Compared to the controls, respectively, *A. gangetica* (10.00%), *A. gangeticus* (1.33%), *L. sativa* (10.00%), *E. crusgalli* (13.33%), and *S. bicolor* (0.67%). Dewi et al. (2017) reported the content of phenolic compounds found from the extraction of a nutsedge, i.e.; 2-firanmethanol; 1,4-benzenediol; 2-methoxy-4-vinylphenol; phenol; 2,6-dimethoxy; vanillic acid; syringic acid; and 3-hydroxybenzoic acid.

Phenolic compounds can inhibit plant growth and even cause tissue death, this results in the process of cell division and elongation being inhibited, then inhibits the germination and growth process [12]. Previous research reported that giving nutsedge powder increased the percentage of abnormal sprouts [13].
3.4. Growth rate

The results of variance showed that the application of bioherbicides made from nutsedge extract had an effect on the percentage of growth speed at the dosage level of *L. sativa*, but had no effect on the other four test weeds. The average growth rates of *A. gangetica* (1.67 - 2.45% Etmal-1), *A. gangeticus* (1.93 - 2.24% Etmal-1), *L. sativa* (2.75 - 4.30% Etmal-1), *E. crusgalli* (1.43 - 1.86% Etmal-1), and *S. bicolor* (0.83 - 1.62% Etmal-1).

Table 4. The average growth rates of *A. gangetica*, *A. gangeticus*, *L. sativa*, *E. crusgalli*, and *S. bicolor*

| Treatment | Treatment | Growth Rate (% Etmal-1) |
|-----------|-----------|-------------------------|
| **Dosage (Kg ha⁻¹)** |          | **A. gangetica** | **A. gangeticus** | **L. sativa** | **E. crusgalli** | **S. bicolor** |
| 45.00     | 2.17      | 2.10                   | 4.30 a              | 1.79           | 1.17           |
| 67.50     | 2.04      | 2.08                   | 2.75 b              | 1.69           | 1.37           |
| **Time**  |          |                        |                      |                |                |
| 2 DBP     | 2.12      | 2.24                   | 3.24                | 1.86           | 0.83           |
| 0 DAP     | 2.17      | 1.93                   | 3.10                | 1.81           | 1.24           |
| 2 DAP     | 1.67      | 2.07                   | 4.22                | 1.86           | 1.38           |
| 4 DAP     | 2.45      | 2.12                   | 3.55                | 1.43           | 1.62           |
| Air       | 4.52      | 4.57                   | 6.05                | 3.00           | 3.38           |

Note: Based on the DMRT at the 5% level, the numbers accompanied by different letters in the same column show significantly different. DBP; the day before planting, DAP; days after planting.

Table 4 showed that the dosage of bioherbicide made from nutsedge powder consistently inhibited the best growth rate at a dosage of 67.50 kg ha⁻¹, except for the growth rate of *S. bicolor*. This showed that higher the dosage is given the more effective in inhibiting the growth rate. This strengthens the results of previous research which reported that giving nutsedge powder extract was able to reduce the percentage of seeds that germinated and inhibited growth speed [13]. The higher the growth rate, the higher the vigor index of a seed [14].

4. Conclusion

The application of bioherbicide made from nutsedge powder with pellet formulation two days before planting to four days after planting was effective in inhibited germination and growth of all tested weeds. The effective application time for almost all tested weeds between -2 to 4 DAP showed no significant difference. The dosage of 67.50 kg ha⁻¹ did not show a significant difference with the dosage of 45.00 kg ha⁻¹ of nutsedge powder in most of the tested weed growth variables.

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