Original Article

Effects of Ultra High Dilution of Paraquat 30 cH on Growth, Chlorophyll Content and Yield of Rice Crop

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Abstract

The application of synthetic fertilizers reduces the natural fertility of the soil and contaminates groundwater. Some photosynthesis inhibitors at ultra-high dilution (UHD) increase photosynthesis, growth, and yield of crops. A weedicide Paraquat at UHD enhanced the growth and yield of potatoes in fields. The objective is to see whether the UHD of Paraquat is also effective on rice. This weedicide was serially diluted with distilled water and manually succussed in 30 steps following the preparation of homeopathic dilutions called potencies. In this way, the 30th potency of Paraquat called Paraquat 30 cH was prepared and preserved in 90 % ethanol. Paraquat 30 cH was diluted with water 1:1000 (v/v) and sprayed on rice plants in a field measuring 0.3125 acres. The control plot of the same area was situated 300 meters away from the test plot. Three treatments were given at an interval of 7 days. The treated plot showed increased growth, chlorophyll content, and rice yield significantly compared to control. The UHD of the weedicide produced precisely the opposite effect of the crude material on plants. The increased growth and yield of rice by Paraquat 30 cH may be due to the enhancement of photosynthesis of treated plants. The UHD of Paraquat increased the yield of rice by 19.35% over the control.

Keywords: Weedicide, potentization, field trial, paddy, increased productivity.

Introduction

In order to boost crop yield, synthetic fertilizers are extensively applied, about 181.9 million Metric tonnes in the world [1]. They affect the quality of soil and its microbial properties [2-3]. They also weaken the shoot and root system of crops and reduce their nutrient value [4]. Ammonium nitrate is used as a synthetic nitrogen fertilizer. It is very much toxic to humans creating malfunction of the liver and kidneys [5]. Here comes the need to find an alternative to these fertilizers. A photosynthesis promoter would be a suitable alternative. We have already observed that some weedicides and drugs at UHD promote photosynthesis and increase about 20% yield of crops [6-9]. We have also observed that Paraquat, a weedicide at UHD, enhances the growth and yield of potatoes [10]. The objective of the present study is to see whether the UHD of Paraquat could enhance the growth and yield of rice.

Rice has been selected because it is a staple food, mainly in Asia, Africa, and Latin America. About 520 million people consume rice in Asia [11]. The three countries, which are the topmost in rice production, are China (148.5 million Metric tonnes), India (116.42 million Metric tonnes), and Indonesia (46.7 million Metric tonnes) [12].

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Materials and methods

Experimental design

Two plots, one control and another treatment were selected for testing the effect of Paraquat 30 cH on rice plants. The drug was applied thrice at an interval of 7 days on rice plants by foliar spray. In order to assess growth and yield, the following parameters were measured for each plot just before each treatment. i) Height of the plants, ii) Number of leaves/plant, iii) Number of tillers /plant, iv) Chlorophyll content in leaves, and v) Total amount of paddy produced. Plants were randomly selected for assessing parameters (i) to (iv). The sample size for parameters (i) to (iii) is 15, and that for the parameter (iv) is 5. The plots belonged to the farmers, who were allowed to continue their regular agronomic practices. The randomized block design used in the field trial could not be applied here because the drug spay would contaminate the adjacent subplots through the air.

Location of the plots

Two plots were situated at Mouza Joyrambati, Anchal Sihar, Block Kotulpur, District Bankura, and State West Bengal, India. The distance between the control and treated plots is 300 meters. Each plot measures 10 Katha (10.65 Decimal or 0.3125 Acre [1 Acre (ac) = 32 Katha.]). Control plot number is 304 (Latitude: 22°55′43″N, Longitude: 87°03′48″E). The treated plot number is 310 (Latitude: 22°55′42″N, Longitude: 87°03′51″E).

Preparation of drugs

Paraquat, a photosynthesis-inhibitor, was purchased from a weedicide dealer from Burdwan, West Bengal. This water-soluble chemical was diluted with distilled water 1:100 and succussed ten times over a pad to prepare the first potency called 1 cH. This potency was further diluted and succussed 29 times in the same process to prepare the 30th potency, Paraquat 30 cH. This final potency was prepared in 90% ethanol and stored at room temperature in the laboratory. The efficacy of an aqueous potency deteriorates in a short time, and for this, it is preserved in 90% ethanol for long-term storage [13-14]. Paraquat 30 cH was diluted with ordinary drinking water 1:1000 just before applying paddy plants in the field.

Cultivation of paddy plants and agronomic practices by farmers

Paddy seeds were purchased from a local market at Burdwan, West Bengal. The seeds are the products of Annapurna Seeds & Farms, Warangal District, Telangana, India. The variety is MTU-7029. The seeds were sown in a nursery plot on 15th June 2019. When the plants grew, they were transplanted from the nursery plot to the test plots on 17th July 2019. After seven days, an amount of 2.5 kg potash mixed with a herbicide (Refit) was applied uniformly on each test plot. Thirteen days later, plants in each test plot were sprayed with an insecticide, Profex Super (Profenofos 40% + Cypermethrin 4%). This insecticide was mixed with water at the rate of 30 ml / 16 L water. After ten days, fertilizer was applied to each test plot at the rate of 5 kg/plot. The fertilizer was a mixture of 2.5 kg DAP and 2.5 kg Urea. After ten days, a systemic fungicide, MASS PLUS (Haxaconazole 5% SC), was mixed with water at the rate of 25 ml /16 L water applied on the plots. Ten days later, an insecticide, KORANDA 505 (Chlorpyrifos 50% + Cypermethrin 5%), was applied. This insecticide was mixed with water in the proportion of 30 ml / 16 L water and applied to the plots. A systemic herbicide, SUMI GOLD, was sprayed on the plants at the same time. It was mixed with water at the rate of 10 ml /16 L water. After 13 days, a mixed fertilizer containing 2.5 kg potash, 1.2 kg DAP and 1.2 kg Urea was applied on each test plot. After 22 days, a contact fungicide, ANTRACOL (Propineb
70% WP), and an insecticide, KORANDA 505, were applied to each test plot. Fungicide and the insecticide were mixed with water at the rate of 10 g / 16 L and 30ml / 16 L, respectively. All the pesticides were obtained from the market in barrels, each containing 16 L water solution or suspension. Two barrels were applied on each plot. S.C = Suspension Concentrate, WP = Wettable Powder.

**Treatment with the test potency**

Paraquat 30 cH mixed with water (1:1000 v/v) was sprayed on paddy plants of one test plot on three sunny days, 15\textsuperscript{th} September 2019, 22\textsuperscript{nd} September 2019, and 30\textsuperscript{th} September 2019. The drug treatment was so designed as to avoid regular agronomic practices followed by farmers.

**Data on plant growth**

Measurements relating to height (cm) of the plants, number of tillers, and leaves per plant were taken just before each treatment (1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd}) in both the untreated and treated plots. Here the 1\textsuperscript{st} measurement was taken one day before the 1\textsuperscript{st} treatment, and the 2\textsuperscript{nd} was taken just before the 2\textsuperscript{nd} treatment. The 3\textsuperscript{rd} measurement was taken just before the 3\textsuperscript{rd} treatment, and the 4\textsuperscript{th} measurement was taken 7 days after the 3\textsuperscript{rd} treatment. The 1st measurement shows the average growth before any treatment. The 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 4\textsuperscript{th} measurements show the effect of the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} treatments, respectively. Fifteen samples for each parameter were taken at random, and their mean and Standard Deviation (SD) were determined separately for each day of measurement.

**Chlorophyll content in leaves**

Leaves of both control and treated plants were collected at random on day 1 (17.09.2019), day 2 (24.09.2019), day 3 (03.10.2019), and day 4 (22.10.2019). The final measurements were taken on day 36 (21.10.2019). Five replicates for each type of chlorophyll a and b were taken and estimated separately by the standard method [8]. The following formula made the quantitative estimation of chlorophylls:

\[
\text{Chlorophyll a} = (13.95 \times A_{665} - 6.88 \times A_{649}) \mu g / ml
\]
\[
\text{Chlorophyll b} = (24.96 \times A_{649} - 7.32 \times A_{665}) \mu g / ml
\]

A=absorbance at the wavelength mentioned in the subscript.

The data are presented in histograms. Appropriate statistics analyzed all the data.

**Statistics**

Using mean height, the mean number of tillers, and the mean number of leaves per plant, we have shown a histogram chart for Day 1, Day 7, Day 15, and Day 36 [Fig (1-3)]. As the sample size is small, we have shown Error Bars as mean ± SD instead of mean ± SE. In order to identify the effect of the drug on plants, we have pooled the data obtained during the 4 successive days of measurements, Day 1, Day 7, Day 15, and Day 36. Then we have calculated the mean, standard deviation, and standard error for both control and treated plants. Using mean chlorophyll a and mean chlorophyll b (n=5), we have shown a histogram chart for Day 1, Day 7, Day 15, and Day 36 [Fig (4, 5)]. As the sample size is small, we have shown Error Bars as mean ± SD instead of mean ± SE. We have pooled the data obtained on Day 1, Day 7, Day 15, and Day 36. We have calculated the mean, standard deviation, and standard error for both control and treated plants for the pooled data.
As the sample size is small and the control plot of the same area is situated near the treated plot, we applied an independent t-test for finding significant difference at a 5% level between the control and treated plants, regarding plant height, number of tillers per plants, number of leaves per plants and chlorophyll content (a, b) in leaves. So, for statistical testing purposes, the Student t probability distribution is followed.

Weather condition during the period of the experiment (15.09.2019-31.10.2019)

Temperature, humidity, rainfall, and brightness of sunshine were recorded for each day. Here only a summary of the weather condition is presented. The maximum and minimum temperature varied between 280°C - 330°C and 200°C-240°C, respectively. Humidity varied between 72% - 99%. Light rainfall occurred on 21.09.2019 (1mm), 27.09.2019 (2mm), 28.09.2019 (1mm), 03.10.2019 (1mm), 06.10.2019 (2mm), 09.10.2019 (4mm), 23.10.2019 (2mm) and 26.10.2019 (2mm). Heavy rainfall occurred on 24.10.2019 (20mm) and 25.10.2019 (22mm). The following days were cloudy: 07.10.2019, 08.10.2019, 17.10.2019, 18.10.2019, and 22.10.2019. All other days were sunny. Water levels in control and treated plot were 12 cm and 11 cm, respectively on 15.09.2019, 1 cm, and 3 cm respectively on 22.09.2019, 13 cm and 14 cm, respectively on 30.09.2019 and 1 cm and 2 cm, respectively on 21.10.2019.

Results

Results are presented in histograms for each growth parameter. Observation on growth parameters was made before each treatment on day 1 (15.09.2019), day 7 (22.09.2019), and day 15 (30.09.2019) in both control and treated fields. An additional observation was made on day 36 (21.10.2019). Plant height showed a significant difference $t = 11.36535$ at 5% level of significance ($P<0.05$) between the control and treated plants (Fig 1). Several tillers/plants showed a significant difference $t = 6.90582$ at a 5% level of significance ($P<0.05$) between the control and treated plants (Fig 2). Some leaves/plants did not show a significant difference $t = 0.93397$ at 5% level of significance between the control and treated plants (Fig 3). Both Chlorophyll a ($t = 12.50036$ at 5% level of significance, $P<0.05$) and chlorophyll b ($t = 3.95114$ at 5% level of significance, $P<0.05$) showed significant differences in amount in the treated plants than the control Fig (4, 5). The difference between the treatment and control regarding plant height, number of tillers, and chlorophyll content appear very small in the case of individual measurement (1st, 2nd, 3rd, or 4th). Nevertheless, it is statistically significant because analyses were done on pooled data rather than individual measurement data. The total amount of paddy produced in control and treated fields was 496 kg and 592 kg, respectively. The difference between the treatment and control was 96 kg. Treatment resulted in a 19.35 % increase in the yield of rice compared to the control. Control and treated fields are shown in Figures 6 A and 6 B, respectively.

Discussion

The chemical formula of Paraquat is C12H14Cl2N2 [15]. The chemical name is 1, 1' Dimethyl-4, 4'-Bityribinium dichloride. The molecular structure is given below:

![Molecular structure of Paraquat](image-url)
Figure 1. Height of rice plants in cm, control left column (black), right treated (grey) before each treatment. Significant difference by t-test (P<0.05) from pooled data of all 4 days taken together.

Figure 2. Number of tillers / plant, control left (black), treated right (grey) before each treatment. Significant difference by t-test (P<0.05) from pooled data.

Figure 3. Number of leaves / plant, left control (black), right treated (grey) before each treatment. No significant difference by t-test at 5% level from pooled data.

Figure 4. Chlorophyll a in leaves, both in control left (black), treated right (grey) before each treatment. Significant difference by t-test (P<0.05) from pooled data.

Figure 5. Chlorophyll b in leaves, both in control left (black), treated right (grey) before each treatment. Significant difference between the control and treatment (P<0.05) from pooled data.

Material doses of Paraquat in the presence of light and oxygen result in the production of hydrogen peroxide, superoxide anion, hydroxyl radical, and singlet oxygen, which are phytotoxic. These chemicals prevent photosynthetic reduction of NADP+. Plasmalema and tonoplast membranes are damaged initially. Later pigments like chlorophyll and thylakoids are broken down. Paraquat primarily inhibits Photosystem-II electron transport and diverts electron flow through photosystem-I. Inhibition of electron transport checks the conversion of absorbed light energy into electrochemical energy. In this way, Paraquat prevents the growth of weeds in a crop field. This toxic weedicide, in contact with soil, undergoes degeneration [10, 16-17]. The rate of photosynthesis depends mainly on factors like the capacity of enzymatic fixation of CO$_2$ into starch and sucrose and photosynthetic electron transport [18]. It is assumed that Paraquat at ultra-high dilution has promoted photosynthetic electron transport and enzymatic fixation of CO$_2$ following the basic principle of homeopathy [19-20]. In homeopathy, nosodes are rarely used for the disease from which they have been produced. In isopathy, the potency is effective on the same patient. However, here the drug is not obtained from the plant, having reduced photosynthesis. So it can be ranked with homeopathy.

Two years ago, we conducted a field trial of Paraquat potency on rice fields but could not get significant results. On analysis of those results, we found that the Paraquat potency was applied on cloudy days when the photosynthetic rate was naturally reduced under meager sunlight. So care must be taken to apply this potency on crops under bright sunlight. The potency has been found effective on two different crops like potato and rice, grown in winter and monsoon. This photosynthetic promoter in fields would bring down the cost of chemical fertilizers and maintain normal soil fertility and natural agro-ecosystem. In this study, it has been mentioned earlier that farmers applied a substantial amount of synthetic fertilizers and pesticides as a routine. The situation would change favorably if unhealthy agronomic practices are reduced to a large extent.

Conclusions

1. Paraquat 30cH increased height, the number of tillers/plant, and chlorophyll content in leaves compared to control.
2. It produced a 19.35 % higher yield of rice compared to the control in a field.

**Figure 6A. Control plot photographed on 15th September, 2019**

**Figure 6B. Treated plot photographed on 15th September, 2019**
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Declaration

The authors declare that there is no conflict of interest concerning the publication of the work.

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