



RESEARCH ARTICLE

# Role of plant growth promoting fungi and doses of chemical fertilizers in improving agronomic response for sustainable wheat crop production

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## Abstract

Plant growth promoting fungi PGPF is considered a major biological technique that results in significant plant growth and yield. Use of high doses of chemical fertilizers increased cost of crop production and environmental pollution. PGPF could be a reliable alternative to reduce application of agrochemicals such as chemical fertilizers in modern agriculture. The present study evaluated the role of these fungi with chemical fertilizer of DAP applied through different doses in agronomic response of production of 2 varieties of Wheat (Ibaa99 and Rasheed) at 2 agricultural seasons 2019-2020-2021. Field plots conducted during 2019-2020 cropping season, to evaluate the role of adding fungal isolate called PGPF-AT with doses of DAP were (0, 50 and 100%) in 0, 120 and 240 Kg ha<sup>-1</sup> respectively, compared with (Commercial Biofertilizer) called Natrusoil as positive control on wheat var. Ibaa99 to improve growth and yield attributes. The study was repeated in the 2020-2021 cropping seasons with var. Rasheed (to discover a variety of effects and confirm results). The results revealed that treatment PGPF-AT was exerted a highly significant increase without differences between doses of DAP 120, and 240 Kg ha<sup>-1</sup> compared to untreated plots for both Ibaa 99 and Rasheed varieties attributes: no. of plants m<sup>-2</sup>, plant height (cm), plant dry weight (g), no. of tillers plant<sup>-1</sup>, no. of spike plant<sup>-1</sup>, spike length (cm), spike weight (g), no. of seeds spike<sup>-1</sup>, weight of 100 grain (g) and grain yield Kg m<sup>-2</sup> in addition to fertilizer use efficiency (Kg Kg<sup>-1</sup>) in both cropping seasons.

## Keywords

*Aspergillus niger*, DAP, Natrusoil, PGPF, Wheat

## Introduction

Some soil microbial, called plant growth promoting fungi PGPF, could be possible alternatives to increase agricultural productivity in low input systems (1). These fungi, which establish a positive interaction with plants in the rhizosphere, play important role in phytoremediation, phytostimulation and bio-fertilization (2). Research results are revealing the various mechanisms by which soil microbes can stimulate plant productivity (3).

In the sustainable agriculture, the PGPF provided a new vision to agro-economy besides their direct benefits by reducing the use of chemical fertilizers and pesticides, as continuous application of these chemicals poses risks to other living things (4).

The system of wheat production in Iraq is recommended to apply about 240 Kg ha<sup>-1</sup> of phosphorus as formulation of DAP (Diammonium phosphate (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> P<sub>2</sub>O<sub>5</sub>:46; N:18), but the farmers apply more than the dosage recommended to increase yield. Using high fertilizer rates as fertilizer application methods, this

may be difficult for smallholder farmers, due to high fertilizer cost.

PGPF, in the sustainable agriculture production system, plays a significant role in increasing phosphorus solubilization and uptake of minerals such as phosphorus (P), nitrogen (N), calcium (Ca), sulfur (S), potassium (K) in addition to micronutrients by plants (5).

Numerous studies have reported that agricultural products can be increased through compensation application of essential chemical fertilizers by using beneficial microbes as bio-fertilizer, also combination of both bio and mineral fertilizers can increase the quality yield in comparison with mineral fertilizers applied alone (6, 7). It was reported that the use of PGPF could be a reliable alternative to reduce the application of agrochemicals such as chemical fertilizer in modern agriculture, because of its harmful effects on humans and the environment (8, 9).

The bio-fertilizer can reduce chemical fertilizer usage by about 20% and improve the restoration of the environment in some cases by leveraging agriculture beside increase crop production. Availablability of many commercial fungal bio-fertilizers are worldwide and consider environmentally friendly and alternative for chemical fertilizers but these products are still used on a very small scale compared to chemical fertilizer compounds (10). It was reported that maximum grain yield was obtained from applied bio-fertilizers treatments: Natrusoil+25% chemical fertilizer CF 4.659 and B1+25% CF 4.691as compared with untreated treatment 3.987 kg ha<sup>-1</sup>(11). This study recommended using a combination of bio and mineral fertilizers to improve grain quality as a reselts of increasing yield by about 17-18%, which will lead to environment conservation achieved without a negative effect.

This study, therefore, aimed to obtain the role of plant growth promoting fungi compare with commercial product of bio-fertilizer as a positive control. Also use their efficiency with chemical fertilizer of DAP applied through two doses in agronomic response of production of 2 varieties of wheat at 2 agricultural seasons.

## Materials and Methods

The study was conducted at the College of Agriculture farm, Wasit University, Iraq (Latitude: 32°30'46" N Longitude: 45°49'05" E). Fungal isolate called PGPF-AT were the best efficiency with wheat in the previous studies by (2, 9) supplied by mycology laboratory, plant protection Dept., College of Agriculture, Wasit university, Iraq, consist of *Aspergillus niger* isolate AD1 and *Trichoderma hamatum* isolate T-113, and Natrusoil (Commercial Biofertilizer compatible mixture of humic acid 1.5% and some *Bacillus* species recorded up to 10 x10<sup>20</sup> c.f.u. /mL.itfrom Vincula Industries, Inc., USA) as a positive control. The PGPF-AT and Natrusoil were used to evaluate the interaction with three levels of phosphate fertilizer (0, 50 and 100%). The mineral fertilizer that was applied is DAP *Diammonium phosphate* (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> P<sub>2</sub>O<sub>5</sub>:46; N:18. The previous elements were used for maximum effect on growth and the yield of 2 varieties of wheat Ibaa99 and Rasheed. The experiment was performed in the first

agricultural season with var. Ibaa99 in field plots during the 2019-2020 and repetition in the 2020-2021 cropping seasons with var. Rasheed to discover variety effect in addition to confirm the results.

## Treatments

The PGPF-AT was inoculated with the sterilized Peatmos named KLASMANN (Germany) and used at a rate of 150 mL/m<sup>2</sup> (2) while used Natrusoil at a rate of 7ml/m<sup>2</sup> based on the manufacturer's recommendation, Both of them were applied evenly on land just before sowing.

The field experiment was conducted in plots measured 10 × 6 m in 3 replicates for each treatment. The treatments were distributed in a randomized completely block design (RCBD). Each plot was irrigated separately to avoid cross-contamination.

Three doses of DAP fertilizer were added to each plot as a source of phosphorus. These doses were (0, 50 and 100% of the recommended dose) in 0, 120 and 240 Kg ha<sup>-1</sup> respectively before planting, according to a recommendation of the national programme for the development of wheat in Iraq. The control treatment was uninoculated and unfertilized with DAP.

A dose of Urea, as a source of nitrogen (200 Kg ha<sup>-1</sup>), was added according to Iraqi agriculture recommendation to all blocks. The plots were planted with wheat seeds var. Ibaa99 during 2019-2020 cropping season and repetition in 2020-2021 season with var. Rasheed to confirm results. The seed was planted after it was treated with sodium hypochlorite 5% for 5 min and washed with sterilized water. The rate of planting was 160 kg seed ha<sup>-1</sup> (12).

## Observations

Application treatments were evaluated for maximum effect on growth and yield at the end of a planting stage. Samples were taken randomly from 1m<sup>2</sup> for each plot in each replicate to measure growth attributes. These attributes were a number of plants m<sup>2</sup>, plant height (cm), plant dry weight (g) and a number of tillers plant<sup>-1</sup>. The yield was evaluated at the maturity stage. Yield attributes were number of spike plant<sup>-1</sup>, spike length (cm) ,Spike weight (g), number of seeds spike<sup>-1</sup>, the weight of 100 grain (g) and grain yield Kg m<sup>2</sup>.

The fertilizer use efficiency (FUE), ability of crops to take up and utilize nutrients for maximum yields, was calculated as the difference in yield (kg ha<sup>-1</sup>) between treatment and the control divided by the amount of fertilizer applied (kg ha<sup>-1</sup>) (13).

$$FUEt = \frac{Yt - Ct}{Ft}$$

Where *FUEt*- represent the fertilizer use efficiency of treatment *t*; *Yt*- the grain yield of treatment *t*; *Ct*- the grain yield of the control treatment, while *Ft*-is the rate of fertilizer used for treatment *t*.

## Data Analysis

All the data was analyzed using GenStat Discovery Edition 4 statistical software and using ANOVA analysis of variance. The

significance of the treatment was determined using an F test followed by L.S.D. test at level ( $P < 0.05$ ).

## Results

The results indicate that in general all growth, yield and grain attributes of wheat varieties Ibaa99 and Rasheed was significantly influenced by the application of PGPF-AT and Natrusoil as well as combined with 3 doses of chemical fertilizer DAP application.

### *Effect of PGPF-AT and Natrusoil, combined with chemical fertilizers on plant growth attributes*

The Data of growth attribute clearly indicated that the treatments exerted a highly significant increase ( $P < 0.05$ ), especially in PGPF-AT application compared to untreated plots for both Ibaa99 and Rasheed varieties in 2019-2020-2021 cropping seasons.

The obtained data (Table 1) distinguishes that the full dose of DAP (240 kg ha<sup>-1</sup>) with PGPF-AT and Natrusoil has a positive effect on wheat growth attributes var. Ibaa99, which is somewhat normal, but the application of PGPF-AT treatment were the highest activity. However, there is no significant difference in the effect between 240 and 120 kg ha<sup>-1</sup> of added doses, *vis.* 21-23.33 for plants/m<sup>2</sup>, 20.94-25.62 g for plant dry weight, 83.53-86.37cm for plant high and a number of tillers plant<sup>-1</sup> were 4.17-4.57 compared to the control treatment were less pronounced were 11-13.67, 12.29-12.99, 67.63-74.73 and 2.47-2.9 respectively in var. Ibaa99 in the 2019-2020 season.

**Table 1.** Growth attributes of wheat var. Ibaa99 with an application of PGPF-AT and Natrusoil in 3 doses of fertilizer DAP in 2019-2020 cropping season

Application Treatment	Fertilizer dose DAP Kg ha <sup>-1</sup>	No. of plants m <sup>-2</sup>	Dry weight Plant g <sup>-1</sup>	Plant high cm	No. of tillers plant <sup>-1</sup>
Control (un-inoculated)	0	7.33	4.08	56.87	2.17
	120	11.00	12.29	67.63	2.47
	240	13.67	12.99	74.73	2.90
PGPF-AT (150 ml m <sup>-2</sup> )	0	17.33	13.34	72.63	2.87
	120	21.00	20.94	83.53	4.17
	240	23.33	25.62	86.37	4.57
Natrusoil (7 ml m <sup>-2</sup> )	0	14.67	9.40	74.30	2.07
	120	16.00	12.53	79.61	3.60
	240	19.67	20.12	82.97	4.30
<b>L.S.D 0.05</b>		<b>1.924</b>	<b>3.830</b>	<b>3.688</b>	<b>0.735</b>

The results (Table 2) were similar in effect for var. Rasheed during the 2020-2021 season, confirms the effect of both PGPF-AT and Natrusoil application compared to control treatment, with the distinction of PGPF-AT treatment under field conditions. With effects of 17.33-19.67 for plants/m<sup>2</sup>, 21-25.82 g for plant dry weight, 83.53-86.37 cm for plant high and a number of tillers plant<sup>-1</sup> were 4.17-4.57 compared to the control treatment were less pronounced were 10.67-12.33, 12.97-13.35, 65.40-74.00 and 2.40-2.83 respectively.

**Table 2.** Growth attributes of wheat var. Rasheed with an application of PGPF-AT and Natrusoil in 3 doses of fertilizer DAP in 2020-2021 cropping season

Application Treatment	Fertilizer dose DAP Kg ha <sup>-1</sup>	No. of plants m <sup>-2</sup>	Dry weight Plant g <sup>-1</sup>	Plant high cm	No. of tillers plant <sup>-1</sup>
Control (un-inoculated)	0	6.33	2.40	59.00	2.10
	120	10.67	12.97	65.40	2.40
	240	12.33	13.35	74.00	2.83
PGPF-AT (150 ml m <sup>-2</sup> )	0	14.67	14.40	70.60	2.76
	120	17.33	21.00	82.23	3.96
	240	19.67	25.82	88.23	4.46
Natrusoil (7 ml m <sup>-2</sup> )	0	12.67	11.56	78.64	2.03
	120	15.00	13.63	80.40	3.30
	240	17.00	22.60	85.13	3.90
<b>L.S.D 0.05</b>		<b>1.809</b>	<b>3.199</b>	<b>4.828</b>	<b>0.387</b>

### *Effect of PGPF-AT and Natrusoil, combined with chemical fertilizers on plant yield attributes.*

The results of treatment effect on yield of wheat Ibaa 99 and Rasheed varieties showed a significant effect ( $P < 0.05$ ) in increasing wheat yield, especially in PGPF-AT application treatment compared to untreated plots in 2019-2020-2021 seasons.

The data of bio-effects of PGPF-AT application treatment on wheat yield attributes in var. Ibaa99 (Table 3) revealed no significant difference in the effect between 240 and 120 kg ha<sup>-1</sup> of fertilizer DAP doses, leading to the highest number of spikes per plant 2.83-3.60, 7.79-9.83 cm for spike length, 5.17-6.34 g for spike weight and 47.07-50.10 for a number of seeds per spike respectively.

The results (Table 4) showed a remarkable effect of

**Table 3.** Yield attributes of wheat var. Ibaa99 with an application of PGPF-AT and Natrusoil in 3 doses of fertilizer DAP in 2019-2020 cropping season

Application Treatment	Fertilizer dose DAP Kg ha <sup>-1</sup>	No. of spike plant <sup>-1</sup>	Spike length cm	Spike weight g	No. seed spike <sup>-1</sup>
Control (un-inoculated)	0	1.03	3.63	2.22	17.77
	120	1.33	5.23	3.52	37.33
	240	1.43	6.10	4.12	43.33
PGPF-AT (150 ml m <sup>-2</sup> )	0	1.40	6.83	3.76	44.83
	120	2.83	7.79	5.17	47.07
	240	3.60	9.83	6.34	50.10
Natrusoil (7 ml m <sup>-2</sup> )	0	1.23	5.87	2.53	31.0
	120	2.07	6.23	3.13	39.30
	240	3.03	8.10	4.37	44.97
<b>L.S.D 0.05</b>		<b>0.315</b>	<b>1.210</b>	<b>0.652</b>	<b>5.224</b>

application treatments on wheat yield attributes in var. Rasheed during 2020-2021 cropping season as there were in var. Ibaa99, compared to control treatment, with the highest effect of PGPF-AT application treatment under field conditions. The data also revealed similar effect between 240 and 120 kg ha<sup>-1</sup> of fertilizer DAP doses,

leading no significant difference, with highest number of spikes per plant 2.70-2.83, 7.56-8.83 cm for spike length, 4.30-5.48 g for spike weight and 46.07-49.43 for a number of seeds per spike respectively.

#### Effect of PGPF-AT and Natrusoil, combined with chemical

**Table 4.** Yield attributes of wheat var. Rasheed with an application of PGPF-AT and Natrusoil in 3 doses of fertilizer DAP in 2020-2021 cropping season

Application Treatment	Fertilizer Dose DAP Kg ha <sup>-1</sup>	No. of spike plant <sup>-1</sup>	Spike length cm	Spike weight g	No. seed spike <sup>-1</sup>
Control (un-inoculated)	0	1.00	3.73	1.92	21.33
	120	1.23	4.66	3.33	30.73
	240	1.33	5.13	3.77	37.90
PGPF-AT (150 ml m <sup>-2</sup> )	0	1.26	6.40	2.42	38.67
	120	2.70	7.56	4.30	46.07
	240	2.83	8.83	5.48	49.43
Natrusoil (7 ml m <sup>-2</sup> )	0	1.16	6.03	2.53	32.67
	120	2.16	6.53	3.20	35.20
	240	2.26	7.83	4.64	44.70
<b>L.S.D 0.05</b>		<b>0.208</b>	<b>0.585</b>	<b>0.475</b>	<b>4.502</b>

#### fertilizers on grain yield and fertilizer use efficiency (FUE)

The efficacy results of used PGPF-AT and Natrusoil on grain yield of wheat Ibaa 99 and Rasheed varieties revealed a significant interaction in increasing grain yield and fertilizer use efficiency (FUE) compared to untreated plots in the 2019-2020-2021 seasons.

The application treatment with PGPF-AT was statistically higher than other treatments, this interaction reflects on grain yield attributes. However, application of 240 and 120 kg ha<sup>-1</sup> doses of fertilizer DAP improved grain yield over control, but without any significant differences between them in the effective application treatment.

The maximum effect (Table 5) was in PGPF-AT application treatment with application of 240 and 120 kg ha<sup>-1</sup> doses of fertilizer DAP *vis.* 4.40-4.43 g and 0.39-0.40 kg m<sup>-2</sup> for the weight of 100 g of grain and grain yield respectively.

In the same sense, the results (Table 6) showed the

**Table 5.** Grain of wheat var. Ibaa99 with an application of PGPF-AT and Natrusoil in 3 doses of fertilizer DAP in 2019-2020 cropping season

Applica-tion Treatment	Fertilizer dose DAP Kg ha <sup>-1</sup>	Weight of 100 g grain	Grain yield Kg m <sup>-2</sup>	FUE Kg Kg <sup>-1</sup>
Control (un-inoculated)	0	2.54	0.07	-
	120	3.16	0.18	3.95
	240	3.53	0.23	2.90
PGPF-AT (150 ml m <sup>-2</sup> )	0	3.90	0.16	-
	120	4.40	0.39	8.27
	240	4.43	0.40	5.12
Natrusoil (7 ml m <sup>-2</sup> )	0	3.32	0.11	-
	120	3.78	0.26	5.68
	240	3.89	0.23	3.95
<b>L.S.D 0.05</b>		<b>0.459</b>	<b>0.053</b>	<b>1.649</b>

corresponding effect of application treatments on wheat grain yield attributes in var. Rasheed during 2020-2021 cropping season with 240 and 120 kg ha<sup>-1</sup> doses of fertilizer DAP *vis.* 4.49-5.18 g and 0.35-0.41 kg m<sup>-2</sup> for the weight of 100 g of grain and grain yield respectively.

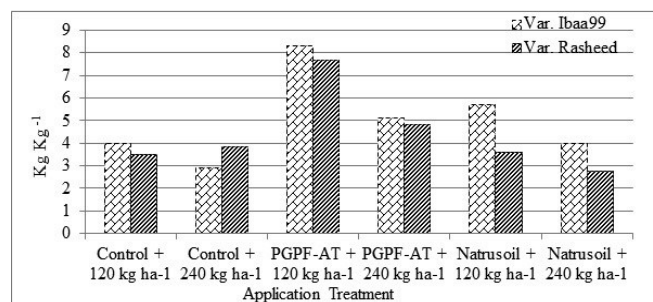
The fertilizer use efficiency (FUE) with 120 and 240

**Table 6.** Grain of wheat var. Rasheed with an application of PGPF-AT and Natrusoil in 3 doses of fertilizer DAP in 2020-2021 cropping season

Application Treatment	Fertilizer dose DAP Kg ha <sup>-1</sup>	Weight of 100 g grain	Grain yield Kg m <sup>-2</sup>	FUE Kg Kg <sup>-1</sup>
Control (un-inoculated)	0	2.45	0.06	-
	120	2.83	0.16	3.46
	240	3.50	0.27	3.83
PGPF-AT (150 ml m <sup>-2</sup> )	0	3.87	0.15	-
	120	4.49	0.35	7.65
	240	5.18	0.41	4.81
Natrusoil (7 ml m <sup>-2</sup> )	0	4.35	0.13	-
	120	3.61	0.23	3.58
	240	3.86	0.28	2.72
<b>L.S.D 0.05</b>		<b>0.690</b>	<b>0.043</b>	<b>1.304</b>

kg ha<sup>-1</sup> doses of fertilizer DAP was decreased with increasing fertilizer rates with significant differences for both PGPF-AT and Natrusoil application treatments (Table 5, 6).

The FUE was higher regarding fertilizer doses in PGPF-AT in both Ibaa99 and Rasheed varieties with 8.27, 5.12 and 7.65, 4.81 kg kg<sup>-1</sup> respectively, in addition to less in Natrusoil were 5.68, 3.95 and 3.58, 2.72 kg kg<sup>-1</sup> respectively (Fig. 1).



**Fig. 1.** Fertilizer Use Efficiency (FUE) with doses of fertilizer DAP for wheat varieties with an application of PGPF-AT and Natrusoil in 2019- 2020-2021 cropping seasons. Samples were taken randomly from 1m<sup>2</sup> for each plot in each replicate, 3 replicates for each treatment.

## Discussion

Using plant growth promoting fungi (PGPF) in improving plant growth with less economic inputs is still a major challenge that would reduce applying of chemicals such as fertilizer. The present study findings are in line with an earlier reported that the beneficial effects of plant growth-promoting fungi (PGPF) and plant growth-promoting bacteria (PGPB) on the growth and yield of wheat under field conditions (14).

Wheat-PGPB (/PGPF)-fertilizers establish several relationships that facilitate beneficial interactions. Several studies confirmed the positive effects of this cooperation by stimulating the proliferation of plant cells, improving the nitrogen-fixation in the roots of wheat, facilitating the uptake of nutrients (15-21).

A higher dramatic yield (5-30%) due to Plant Growth Promoting Bacteria (PGPB) inoculation (22). Reports are pointed out that PGPB significantly increase the grain yield of wheat (16, 23-25). Similarly, it was revealed that the combination of fertilizer and PGPB improved the growth, yield and nutrient use efficiency of wheat (15). Inoculation of wheat using PGPB significantly enhanced grain weight per spike, shoot and root dry weight, shoot and root length, shoot and root nitrogen content (20). It was reported that the combination of PGPB and chemical fertilizers improved wheat agronomic response (plants number, tillers number, spikes number per plant, spike length, spike weight, seeds number per spike and grain yield, grain protein concentration) (18, 19, 26-31).

Treatment inoculated with PGPF will enhance vigor index, seed germination and emergence index in wheat (32, 33). An increase in growth responses of PGPF-treated wheat was detected at different stages (seedling, vegetative, pre-flowering, flowering and seed maturation) of plant growth and development (34). It was reported that a combination of microbes will have multiple positive effects and could be more beneficial for increasing growth and yield and may provide plant protection under different conditions (35).

It was revealed that PGPF augmented the growth and development of shoot in wheat (13, 36). Similarly, soil treatment with PGPF increased dramatically number of seeds per fruit, fruit weight, number of fruits per plant and dry weight of 100 seeds (37). It was also revealed that PGPF contribute significantly to improvement of wheat yields (36, 38). Some previous works have reported the positive effect of PGPF-fertilizer interactions in improving the growth and physiology of wheat plants (39, 40).

Irrespective of differences in agro-ecological conditions in the study sites which inoculated with PGPF, all the fertilizer rates in banding were increased yields compared to uninoculated treatment. This reveals that wheat production is needed for applying fertilizer but low doses. The efficient method of the application that is with a smaller amount of fertilizer is what is most important for farmers. This method will have a high potential to increase farmers' interest besides economic sustainability concerning applying fertilizer to wheat. In this respect, results showed that the half dose of fertilizer application (120 kg ha<sup>-1</sup>) were found to improve wheat growth and yields with smaller quantities of fertilizer full dose (240 kg ha<sup>-1</sup>).

The small dosing of applying fertilizers can be considered as an alternative to what has been recommended through the national extension system. The small dosing application consists of small quantities of fertilizer in the planting station at planting; this will

enhance fertilizer use efficiency and improves yields while minimizing input cost and improving return on investment (41, 42). This indicates that the smallest dosing application rate is more efficient than the highest dosing rate. This advantage by using plant growth-promoting fungi (PGPF) can be improved wheat production either under normal or stress conditions (43).

## Conclusion

The study concludes that wheat growth and yield responses to biofertilizers alone or in combination with 50 % of the fertilizer recommended dose were clearly positive. The biofertilizer increases both growth and yield components of wheat,. Among the benefits of using -PGPF as biofertilizers can be reduction in cost as compared with the use of a full dose of chemical fertilizer. Hence, Efforts should be strengthened to improve the efficacy and commercialization of PGPF.

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## Authors contributions

AH conducted the study and wrote a part of the manuscript. NK analyzed the data and contributed in paper writing. AA supervised the work and approved the final manuscript. AR performed the statistical analysis. The authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest :** Authors do not have any conflict of interests to declare.

**Ethical issues :** None

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