



Effect of nano-particles on growth, productivity, profitability of Indian mustard (*Brassica juncea*) under semi-arid conditions

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Received: 16 July 2018; Accepted: 21 January 2019

ABSTRACT

The field experiment was conducted to study the effect of nano-particles (chitosan and titanium oxide) on growth, yield and economics of Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] during 2013-14 and 2014-15 comprising nine treatments of seed and foliar spray of nano-particles and thiourea in RBD with three replications. Maximum dry matter accumulation was recorded under seed treatment with titanium oxide (STTO) at 1000 ppm during both the years. The SPAD value indicated that higher greenness was noticed with STTO @ 1000 ppm, followed by titanium oxide spray @ 500 ppm. The maximum primary and secondary branches were recorded in STTO@ 1000 ppm, followed by the foliar spray of titanium oxide @ 500ppm. The bold seeds were also produced with higher 1000 seed weight (5.9 gm and 6.1 gm during 2013 and 2014, respectively) in STTO @ 1000 ppm. Across all the treatments, STTO @ 1000 ppm resulted in higher yield attributes. Seed treatment with STTO @ 500 ppm recorded 20% higher seed yield over thiourea. STTO @ 1000 ppm and STCH @500 ppm recorded significantly higher biological yield over rest of the other treatments. Significantly ($P \leq 0.05$) higher oil content was noticed in STTO @ 1000 ppm during both the years and the highest oil yield (852 and 836 kg/ha), highest net return and maximum B:C ratio (2.95 and 2.76) during 2014-15 and 2015-16, respectively) was also recorded in STTO @ 1000 ppm. The pooled analysis of two years data showed that the highest nitrogen, phosphorus and potash uptake from seed and stover was recorded under STTO 1000 ppm, followed by STTO @ 500 ppm and foliar application of chitosan @ 1000 ppm.

Key words: Chitosan, Economics, Nanoparticles, Oil content, Productivity enhancement, Rapeseed-mustard, Titanium oxide

Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] is an ideal crop for resource poor areas, where there is limited scope for cultivation of other crops. Almost 50% area and production of Indian mustard crop is confined to Rajasthan. The area, production and productivity of oilseed in India are 26.2 Mha, 32.1 MT and 1225.0 kg/ha, whereas for rapeseed-mustard are 6.0 Mha, 7.98 MT and 1324.0 kg/ha, respectively (Anonymous 2018). Among oilseed crops comparatively higher productivity gains in rapeseed-mustard are reported due to improved agronomic management. The productivity of Indian mustard can be further enhanced with support of improved agronomic management to the high yielding Indian mustard varieties. It has been widely reported that improved and high potential cultivars of Indian mustard respond well to best management practices

(BMPs), including technical inputs like proper seed and sowing, irrigation water management, judicious nutrient management, weed management etc. (Rathore *et al.* 2014). The BMPs like crop nutrition and irrigation management together can enhance Indian mustard productivity to 2.5 t/ha at national level, which is fluctuating between 1.1-1.3 t/ha. The input requirement of rapeseed-mustard for realizing maximum productivity is comparatively less to cereals. It is also true that precise use of these limited resources at right time led to tremendous improvement in seed yield. There exists a commercially exploitable yield reservoir to the tune of nearly 73% of the national production, which can be harnessed by the adoption of currently available improved technologies (Hegde 2012). This yield gap is huge opportunities to be exploited through best management practices. In this regard, use of nano-particles has huge potential to enhance input use efficiency (Bansal *et al.* 2014). The new or vastly different properties of nano-particles created through manipulation of individual atoms and molecules help in activating the plant system for efficient functioning especially for nutrient, water uptake and developing resistance mechanism against biotic and abiotic stresses. The nano-particles in crop production have been

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designed for slow release of active ingredients and also, the treatment requires application only once throughout the life cycle of the crop. The present study was thus planned to assess the significant effect of nano-particles vis-a-vis their economic viability for rapeseed-mustard crop under challenged agro-ecosystem for ensuring edible oil security in India.

MATERIALS AND METHODS

Experimental site: The experiment was conducted at DRMR (77.3°E longitudes, 27.15°N latitude and 178.37 MSL) during 2013-14 and 2014-15 on Indian mustard var. NRCDR 02. The maximum and minimum relative humidity ranged between 77-98% and 23-74%, respectively, during the crop season. The pan evaporation was maximum during initial and later stage of crop growth, while reached minimum during January due to prevailing low temperature (Table 1).

Layout and the experimental design: The experiment was undertaken during *rabi* season of 2013-14 and 2014-15 in RBD replicated thrice. There were 9 treatments i.e. ST (seed treatment) titanium oxide (ST_{TO}) @500 and 1000 ppm; ST (Seed treatment) Chitosan (ST_{CH}) @500 and 1000 ppm, foliar spray (FS) of TO @500 and 1000 ppm, foliar spray of CH @500 and 1000 ppm and foliar spray of thiourea @0.1 %. Chitosan nano-particles (purity: >99%, 80-150 nm size) and titanium Oxide nano-powder (TiO₂, anatase, 99+%, 10-25 nm) were used as seed treatment and foliar spray of different concentration in Indian mustard. Two irrigations coinciding with critical stages of crop growth were given though check basin at 30-35 and 65-70 days after sowing (DAS). The initial soil pH and EC were 8.5-9.5 and 2.5-3.5 dS/m respectively. The soil was poor in available N (130.0 kg/ha), medium in P₂O₅ (19.5 kg/ha) and K₂O (245 kg/ha).

Crop husbandry: The experiment was conducted under *Sesbania aculeata* green manure-Indian mustard cropping system. Indian mustard variety NRCDR02 of 140-150 days duration was sown on 22nd and 24th October and harvested on 10th and 12th March during 2013-14 and 2014-15, respectively. Across all the treatments, recommended agronomic practices were followed for sowing, irrigation, nutrient, weed management and for plant protection. Thinning of extra plants was done at 20 DAS. For weed management, pre-emergence spray of pendimethalin was

done @1.0l a.i./ha. The plant protection measures were adopted for management of insect-pest (aphid) and diseases (stem rot, white rust and alternaria leaf blight) as standard recommended practices.

Observations: Photosynthesis and gas exchanges activities of mustard were measured using a portable photosynthesis system (Model LI-6400, LICOR, inc. Lincoln, Nebraska USA) during 1000-1300 h under clear sunshine conditions at 40 and 80 DAS and pooled rgw data for two years (Table 2). The fully expanded leaves on two-third above part of plants were selected randomly from each plot. The portable PS system can simultaneously determine the net rate of photosynthesis (Pn), stomatal conductance (gs), intercellular CO₂ concentration (Ci), and transpiration rate (E), etc. The SPAD meter makes instantaneous and non-destructive readings on a plant based on the quantification of light intensity (peak wave length: approximately 650 nm: red LED) absorbed by the tissue sample. The yield attributes and observations were recorded at maturity of the crop by selecting five random plants from each plot. The border of 0.60 m was left for recording seed yield and it was extrapolated to calculate per ha seed yield.

Statistical analysis: The data were statistically analyzed using Fisher's analysis of variance technique (Steel *et al.* 1997) and the treatments means were compared by Duncan's Multiple Range (DMR) test at level of 0.05 probabilities. The standard analysis of variance (ANOVA) was performed using SPSS 17.0 statistical software to compare the treatment means for each year separately.

RESULTS AND DISCUSSION

Growth, photosynthesis and yield attributing characters: Use of nano-particles through seed treatment (ST) and foliar spray (FS) significantly influenced ($P \leq 0.05$) plant height, dry matter accumulation, photosynthetic rate, transpiration and days to flowering (Table 2). ST_{TO} @1000 ppm had resulted in higher plant height (182 cm). Nano-particles titanium oxide (TO) was found to be superior over chitosan and thio-urea in all combinations. Similar trend was found with respect to dry matter accumulation and SPAD values. Maximum dry matter accumulation was recorded under ST_{TO} @ 1000 ppm during both the years but relatively lesser dry matter per plant was recorded during 2014-15. The SPAD value indicates N status of plant and also the

Table 1 Prevailing weather conditions during crop growth period 2013-15 and 2014-15

Month	2013-14				2014-15			
	Temp (°C)		Rainfall (mm)	Pan evaporation (mm/day)	Temp (°C)		Rainfall (mm)	Pan evaporation (mm/day)
	Max.	Min.			Max.	Min.		
October	32.1	20.1	30.8	3.2	34.6	18.9	2.4	3.6
November	27.9	11.1	6.0	2.4	30.2	12.0	0.0	2.0
December	22.4	7.7	13.3	1.4	20.6	6.2	0.0	1.2
January	17.0	7.4	55.7	0.6	16.9	7.4	39.3	0.4
February	21.6	8.5	10.4	1.5	25.9	10.5	0.0	2.1
March	29.4	13.2	13.8	3.2	28.7	14.9	57.0	2.3

Table 2 Effect of nano particles on growth parameters of Indian mustard

Treatment	Plant height (cm)		Dry weight (g/plant)		SPAD (30 DAS)		Photosynthetic Rate ($\mu\text{mol}/\text{m}^2/\text{S1}$)		Rate of transpiration ($\text{mmol}/\text{m}^2/\text{S1}$)		Days to 50% flowering	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
ST Titanium oxide @500 ppm	179b	179b	40.0a	39.5b	45.0c	44.6c	8.5c	8.2b	22.3bc	21.8a	57.2	56.8
ST Titanium oxide @1000 ppm	182a	189a	50.0a	45.6a	54.2a	55.2a	12.3a	12.4a	24.6d	25.2d	61.2	62.0
ST Chitosan @500 ppm	168c	172b	36.7a	32.6c	48.2b	48.2b	10.5b	11.1a	23.1c	22.1a	60.2	59.6
ST Chitosan@1000 ppm	169c	173b	36.7a	36.2bc	49.2b	48.5b	8.9c	9.0b	20.3b	21.7a	58.2	58.0
Foliar spray of titanium oxide @500 ppm	173b	173b	36.7a	34.5c	47.5c	47.5b	7.8c	7.9bc	19.2a	21.2.2b	58.3	57.9
Foliar spray of titanium oxide @1000 ppm	165b	165c	41.7a	40.2b	49.2b	48.6b	8.4c	8.3b	17.5a	18.1a	59.1	59.0
Foliar spary of chitosan @500 ppm	173b	173b	26.7a	28.5d	45.2c	43.6c	7.6c	7.7c	19.2b	19.1b	57.5	58.0
Foliar spray of chitosan @1000 ppm	174b	174b	40.0a	38.6b	46.5c	46.1bc	8.4c	8.2b	20.8c	21.1c	59.2	58.7
Thiourea @0.1 %	170b	170b	40.0a	38.2b	46.8c	46.2bc	8.6c	8.3b	19.6a	19.5b	56.2	57.2

chlorophyll content of the leaves and a higher greenness was noticed under ST_{TO} @ 1000 and @ 500 ppm (Table 2). Nano-titanium oxide enhances the antioxidant activities of catalase, peroxidase, superoxide dismutase activity which protect the chloroplast from excessive light and there by improves the chlorophyll activity (Hong *et al.* 2005). A higher SPAD value also coincided with higher rate of photosynthesis. Maximum photosynthetic rate was in ST_{TO} @ 1000 ppm during both the years. With higher rate of photosynthesis, increased transpiration was also recorded in FS_{TO} @1000 ppm. Supplementation of titanium oxide stimulates chlorophyll formation (recorded as higher SPAD) and enhances Rubisco activity (Yang *et al.* 2006). The higher plant growth and development with supplementation of titanium oxide is substantiated by increase in photosynthetic pigment, and total soluble leaf protein content (Raliya *et al.* 2015). The ST_{TO} @ 1000 ppm resulted in relatively longer days to flowering (61.2 and 62.0 days). Prolonged days to commencement of reproductive stage ensure more time for source to produce higher photosynthates and ultimately higher seed productivity. According to Giraldo *et al.* (2014), nano-particles have unique physico-chemical properties and the potential to boost the plant metabolism. Giraldo *et al.* (2014) and Torney (2007) have also reported ability of nano-particles to enter into plants cells and leaves which accelerate growth and development. However, efficacy of nano-particles depends on their concentration and the response varies from plants to plants (Siddiqui *et al.* 2015). Mahmoodzadeh *et al.* (2013) have also reported enhanced seed germination and growth of canola seedlings and seed productivity due to application of nano-titanium oxide.

The yield attributes of Indian mustard, viz. primary branches, secondary branches, seeds/silique, total silique/plant and 1000 seed weight were significantly ($P \leq 0.05$)

influenced by use of different combination of nano-particles. The maximum numbers of primary and secondary branches were in ST_{TO} @ 1000 ppm followed by the FS_{TO} @ 500 ppm (Table 3). These branches contain main yield attributing characters, bear the silique and to large extent determine the boldness of the seed and also the oil content. The seed per silique and total siliquae were recorded maximum in ST_{TO} @ 1000 ppm. The higher the silique and seed number per silique will have positive impact on seed yield. The bold seed were also produced with higher 1000 seed weight (5.9 g and 6.1 g) in ST_{TO} @ 1000 ppm. These important yield attributing characters lead to positive trend for enhanced seed yield. Silique length, number of seeds/silique and silique number varied depending upon the type of branches. Across all the treatments, ST_{TO} @ 1000 ppm resulted in higher silique length and also the total number of silique on primary branches. The number of seeds/silique were higher on main shoot compared to silique number on primary and secondary branches, resulting in higher yield. The yield improvements under water deficit stress condition by use of titanium oxide have been also reported (Jaberzadeh *et al.* 2013). More number of primary branches impels higher silique length and seeds/silique due to seed treatment with titanium oxide. Efficacy of nano-particles due to different concentration, chemical composition, size, surface covering, reactivity and methods of applications was also reported by Khodakovskaya *et al.* (2012).

Seed, oil yield, harvest index and Economics: The impact of different combination of nano-particles and thiourea were evident on seed and oil yield. The ST_{TO} @1000 ppm produced significantly higher ($P \leq 0.05$) growth and yield attributing parameters which improved the final seed yield. However, ST_{TO} @ 500 ppm resulted in significantly lower seed yield during both the years (Table 3). This

Table 3 Effect of nano particles on yield attributes of Indian mustard

Treatment	Primary branches		Secondary branches		Seeds/siliquea		Total siliquea		1000 seed weight (g)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
ST Titanium oxide @500 ppm	3.5a	3.3bc	2.46ab	5.1a	11.8b	14.1ab	199.4b	190.2a	5.1a	5.1b
ST Titanium oxide @1000 ppm	4.3b	4.6a	6.6d	5.9a	13.0a	15.7a	228.7a	195.2a	5.9a	6.1a
ST Chitosan @500 ppm	3.1a	3.1c	3.40b	4.6a	10.4c	14.2a	164.0c	157.5c	4.6a	4.6c
ST Chitosan @1000 ppm	3.8a	3.6b	4.40ab	5.1a	10.6c	13.7b	176.9c	154.6c	5.1a	5.1b
FS of titanium oxide @500 ppm	3.8a	3.5b	4.60c	4.6a	11.6b	13.6b	167.9c	161.7c	4.6a	4.6c
FS of titanium oxide @1000 ppm	3.9a	3.9a	5.06c	5.2a	11.5b	14.1ab	170.3c	185.2b	5.2a	5.2b
Foliar spary of chitosan @500 ppm	3.9a	3.9a	3.10b	5.9a	11.1b	14.7ab	165.0c	167.6c	5.6a	5.4b
Foliar spary of chitosan @1000 ppm	3.9a	3.9a	2.80b	5.1	10.8c	14.6ab	176.2c	143.1d	5.1a	5.1b
Thiourea @0.1 %	3.4a	3.4bc	1.66a	5.1a	11.1b	14.7ab	178.6c	161.1c	5.1a	5.1b

Note: FS foliar spay, ST-Seed treatment, Within a column, values represented with different lower-case letters indicate significant differences (P=0.05).

suboptimal concentration probably failed to stimulate the physico-biochemical system of the plants to the extent which is minimally required. During 2014-15, the trend in seed yield was slightly flouting due to the fact that response to other nano-particles (ST_{TO} @ 1000 ppm) with varied concentration was significantly higher under FS_{CH} @500 ppm and 1000 ppm. However, during 2014-15, ST_{TO} @ 500 ppm resulted in overall 21% higher seed yield and it was 30% higher over ST_{CH} @ 500 ppm and 1000 ppm. The ST_{TO} @ 500 ppm recorded 20% higher seed yield over thiourea. But the foliar spray FSTO @ 1000 ppm was detrimental in terms of lowering the seed yield and biological yield of Indian mustard (Table 4). The ST_{TO} @ 000 ppm and chitosan @ 500 ppm was recorded significantly higher biological yield over rest of the other treatments. Qi *et al.* (2013) reported that exogenous application of titanium oxide as nano-particle also improves net photosynthetic rate, water conductance and transpiration rate in plants. The negative response of chitosan and titanium oxide on seed yield was noticed but its response was relatively lesser in biological yield. The conversion of vegetative

biomass into seed yield was better with FS_{CH} @ 500 and 1000 ppm, which reflected in significantly higher harvest index. Significant variation was noticed in oil yield under treatments. Nano-titanium oxide improved seed yield due to the photo-catalyst ability of nano sized titanium oxide which led to increase in photosynthetic rate. It also regulates enzymes activity involved in nitrogen metabolism such as nitrate reductase, glutamate dehydrogenase, glutamine synthase and glutamic-pyruvic transaminase. This helps to the plants to absorb nitrate and also favours the conversion of inorganic nitrogen to organic nitrogen in the form of protein and chlorophyll that could increase crop productivity (Yang *et al.* 2006, Mishra *et al.* 2014).

Production efficiency (13.96 and 13.7 kg/ha/day during 2013 and 2014, respectively), net return, B:C ratio and profitable efficiency were significantly higher under ST_{TO} @ 1000 ppm. Similarly, FS_{CH} @ 500 ppm and 1000 ppm also recorded higher production efficiency. Water use efficiency in terms of monetary return was highest in ST_{TO} @ 1000 ppm and lowest was observed in FS_{TO} @ 1000 ppm. Highest net return (₹ 62,226 and 58,374 during 2013

Table 4 Effect of nano particles on seed, biological yield and harvest index of Indian mustard

Treatments	Seed yield (kg/ha)		Biological yield (kg/ha)		Harvest index		Oil yield (kg/ha)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
ST Titanium oxide @500 ppm	1672 ^a	1688 ^{bc}	6222 ^c	6572 ^b	0.28 ^a	0.26 ^b	706 ^b	677 ^b
ST Titanium oxide @1000 ppm	2011 ^b	2033 ^a	7344 ^a	7450 ^a	0.28 ^a	0.27 ^b	852 ^a	836 ^a
ST Chitosan @500 ppm	1524 ^a	1561 ^{ab}	7090 ^b	5482 ^c	0.22 ^b	0.28 ^b	646 ^c	632 ^b
ST Chitosan @1000 ppm	1630 ^a	1543 ^{ab}	6222 ^c	5337 ^c	0.26 ^{ab}	0.28 ^b	690 ^b	622 ^b
FS of titanium oxide @500 ppm	1545 ^a	1616 ^b	6265 ^c	6535 ^b	0.25 ^b	0.24 ^{bc}	652 ^c	659 ^b
FS of titanium oxide @1000 ppm	1587 ^a	1380 ^c	6265 ^c	6590 ^b	0.25 ^b	0.21 ^c	672 ^{bc}	566 ^c
FS of chitosan @500 ppm	1524 ^a	1979 ^b	6159 ^c	6209 ^b	0.25 ^b	0.32 ^a	642 ^c	811 ^a
FS of chitosan @1000 ppm	1566 ^a	1943 ^b	6053 ^c	6263 ^b	0.26 ^{ab}	0.31 ^a	665 ^c	798 ^{ab}
Thiourea @0.1 %	1660.2 ^a	1688.4 ^{bc}	5854 ^c	5809 ^c	0.27 ^a	0.29 ^b	609.5 ^d	689 ^{ab}

Note: FS foliar spay, ST-Seed treatment, Within a column, values represented with different lower-case letters indicate significant differences (P=0.05).

Table 5 Effect of nano particles on production efficiency and economics of Indian mustard

Treatments	Production efficiency (kg/ha/day)		NR (₹/ha)		B:C ratio		Profitable efficiency (₹/ha/day)	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
ST Titanium oxide @500 ppm	11.61 ^a	11.4bc	48682 ^c	50236bc	2.32 ^e	2.39ef	338.1 ^c	339.4
ST Titanium oxide @1000 ppm	13.96 ^b	13.7d	62226 ^d	58374c	2.95 ^f	2.76f	432.1 ^d	415.0
ST Chitosan @500 ppm	10.57 ^a	10.5ab	46742 ^{bc}	43066b	2.23 ^e	2.05de	324.6 ^{bc}	291.0
ST Chitosan @1000 ppm	11.30 ^a	10.4ab	47191 ^{bc}	41902b	2.23 ^e	1.98cde	327.7 ^{bc}	283.1
FS of titanium oxide @500 ppm	10.71 ^a	10.9ab	39086 ^{ab}	42062b	1.46 ^c	1.58bc	271.4 ^{ab}	284.2
FS of titanium oxide @1000 ppm	11.04 ^a	9.3a	34377 ^a	29026a	1.05 ^{ab}	0.88a	238.7 ^a	196.1
FS of chitosan @500 ppm	10.57 ^a	13.4cd	37124 ^a	51156bc	1.33 ^{bc}	1.83cd	257.8 ^a	345.6
FS of chitosan @1000 ppm	10.87 ^a	13.1cd	31097 ^a	47748b	0.89 ^a	1.24ab	216.0 ^a	322.6
Thiourea @0.1 %	10.13 ^a	11.4bc	39424 ^{abc}	43212b	1.86 ^d	2.25de	273.8 ^{abc}	292.0

Note: FS foliar spray, ST-Seed treatment, Within a column, values represented with different lower-case letters indicate significant differences (P=0.05).

and 2014, respectively) was obtained in ST_{TO} @ 1000 ppm and it produced 22 and 14% higher net return over ST_{TO} @ 500 and 1000 ppm, respectively. Similarly, foliar application of titanium oxide and chitosan was not as effective as seed treatment. Lowest net return (₹ 29,026) was recorded from FS_{TO} @ 1000 ppm during 2013-14, but FS_{CH} @ 500 and 1000 ppm resulted in statically at par net return to other treatment except ST_{TO} (1000 ppm). Benefit-cost ratio was >2.0 under seed treatment with titanium oxide and chitosan @ 500 and 1000 ppm and maximum B:C ratio (2.95 and 2.76 during 2013 and 2014, respectively) was noticed in ST_{TO} @ 1000 ppm due to poor yield and net return. Profitability was also significantly higher in ST_{TO} @ 1000 ppm, which was ₹ 432.0 and 415 per day during 2013 and 2014, respectively (Table 5). Govorov and Carmeli (2007) believed that the metal nano-particles can induce the efficiency of chemical energy production in photosynthetic systems and WUE. Titanium oxide nano-particles are economic to synthesize, have easy down stream processing and are environmentally safe. They may, thus act as nano-nutrient fertilizer to enhance crop production by stimulating plant metabolic activities.

Nano-titanium oxide as seed treatment @ 1000 ppm resulted in significant improvement of growth, yield attributes, seed and oil productivity of Indian mustard over chitosan. Also, the seed treatments with titanium oxide and chitosan were more advantageous over foliar spray. The higher net returns, B:C ratio and over-all profitability was also obtained from use of nano-particles in mustard crop. Use of nano-particles in crop production could help in achieving higher precision in use of the technical inputs. At the same time, it can also address the major challenge of low productivity and poor resource use efficiency. The enhanced production efficiency, profitability and monetary water use efficiency can be achieved with use of nano-particle like titanium oxide in Indian mustard under semi-arid conditions. Results from the present study, however, need to be standardized under different agro-ecological conditions.

ACKNOWLEDGMENTS

The authors are highly thankful to the Director, ICAR-Directorate of Rapeseed-Mustard Research for providing all the logistic to carry out the experiments. The authors are grateful to the technical and supporting staff who helped in carrying out field operations and recorded observations timely.

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