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Application of *Trichoderma* Spp. in Controlling *Orobanche Ramosa* Parasitism in Chamomile

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ABSTRACT
Biological control could play a role in the management of *Orobanche* parasitism in plant production. Two greenhouse experiments were conducted to evaluate the effect of three *Trichoderma* spp.including *T. hamatum*, *T. viride* and *T. harzianum* on controlling *O. ramose* parasite in chamomile plant .Soil treatment with *Trichoderma* (at 3.6×10^{8} propagules g^{-1}) was effective in reducing infection with *O. ramose* by delaying in *Orobanche* attachments and reducing number and growth of tubercles. *T. hamatum* and *T. viride* completely protected chamomile plants against *Orobanche* infestation until 3 months after transplanting. Fungi treatments caused more than 75% reduction in number of *O. ramosa* attachments, fresh and dry weights of tubercles. Moreover, *Trichoderma* treatments improved chamomile growth, where the treatments increased fresh and dry plant biomass between 59-90% and 67.6-112.2%, respectively compared with untreated plants. Among the three applied fungi, *T. viride* produced the most enhancement effects on chamomile growth. Results revealed that the application of tested *Trichoderma* spp. could play an important effect in controlling *O. ramosa* parasitism in chamomile as a natural bio-herbicide as well as enhancing the growth parameters of chamomile.

Keywords: *Matricaria chamomilla* L., holoparasite, fungi, bio-herbicide

1. Introduction

Chamomile plant (*Matricaria chamomilla* L.) is grown in Egypt and it is used for medical and industrial purposes. *Orobanche* species are serious parasitic weeds causing considerable losses in many major crops including chamomile. *Orobanche* attack plant roots of many agricultural crops, *i.e.* cabbages, potato, tomato, melon, watermelon and other crops in the Mediterranean region (Amsellem *et al*., 2001; Hassan *et al*., 2004; Nawar and Sahab, 2011).

Orobanche (broomrape) is widespread on winter crops as well as recently become one of the most serious problems in Egyptian agricultural productions (Abdel-Kader and El-Mougy, 2007). No single effective and economical method for management of *Orobanche* is available, where alternative methods *viz.* preventive, physical, chemical, agronomic, biological, crop resistance and integrated methods are needed to manage this parasite (Habimana *et al*., 2014; Punia, 2014). Applications of biological agents become play an important role for controlling plant pathogens as well as weeds in fields. Because of application of herbicides has many problems such as contamination of groundwater, destruction to the non-target species and induction of resistance against herbicides in numbers of weed species as well as other control methods become more unsuitable where the weeds are widespread. Therefore, the researchers moved toward biological control as an alternative approaches for managing of weeds (Mustafa *et al*., 2019).

The capability of *Trichoderma* spp. *i.e. T. harzianum* and *T. viride* for controlling *Orobanche* spp. in peas, faba bean and tomatoes under field conditions was observed by Abdel-Kader and El-Mougy (2009). They found that soil treatment with these fungi, as bio-control agents, was effective in reducing broomrapes infection and increasing yield of plants. Also, Abdel-Kader and El-Mougy (2007) found

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that application of *T. harzianum* and *T. viride*, followed by foliar spray with glyphosate herbicide was the most appropriate treatment for controlling *O. ramosa* in tomato plants under field conditions. The treatment significantly reduced the incidence and intensity of broomrape branches as well as increased the tomato yield in comparison with the individual treatment. It is revealed that this treatment could develop because of an effective, applicable and cost-effective method acceptable for controlling *O. ramosa* in tomatoes. Application of *T. harzianum* and the commercial product (Plant Guard, *T. harzianum*) reduced number of *Orobanche* shoots in comparison with the control. *T. viride* and *T. hamatum* were found to be effective in reducing the *Orobanche* shoot numbers (Nawar and Sahab, 2011). Field application of *T. harzianum* alone or in combination with *Bacillus megatherium* var. *phosphaticum* significantly reduced number of *O. crenata* emergence and significantly increased faba bean biomass such as pod/plant, grain yield and 100 seed weight, compared to the controls (Yahia *et al*., 2018). *T. harzianum*, *T. viride* and *T. viern*s could protect faba bean plants against *O. crenata* infection, where the treatments reduced number, fresh weight and dry weight of juveniles. All *Trichoderma* spp. treatments highly increased the plant growth parameters. It is clear that application of *Trichoderma* spp. could play an important role in controlling broomrape in faba bean as a natural bio-herbicide (El-Dabaa and Abd-El-Khair, 2020).

Therefore, this study was conducted during two successive seasons to determine the effect of *orobanche* infestation on chamomile growth and the efficacy of *Trichoderma* spp. i.e. *T. hamatum*, *T. harzianum* and *T. viride* on controlling *O. ramose* infestation and improving chamomile growth.

2. Materials and Methods

2.1. Plant materials

Seeds of chamomile (*Matricaria chamomilla*) were obtained from Ministry of Agriculture, Giza, Egypt. Seeds were firstly sown in a seedling tray (50 x 90 cm diameter).Then, after 30 days of sowing; seedlings were transplanted in experimental pots. Seeds of *O. ramosa* or *O. crenata* were collected from an infested field and stored in the dark at 25°C until use. Three of *Trichoderma* spp., *i.e. T. hamatum*, *T. harzianum* and *T. viride*, were obtained from Pest Rearing Department, Central Agricultural Pesticides Laboratory, Agricultural Research Centre, Dokki, Giza, Egypt (El-Dabaa and Abd-El-Khair, 2020).

2.2. Preparation of Trichoderma spp. inocula

For preparation of *Trichoderma* spp. propagules; each of *T. hamatum* or *T. harzianum* or *T. viride* was separately prepared using Sorghum - Sand - Water (2:2:1, V: V: V) medium. The sterilized medium was individually inoculated by each fungus, using fungal disc (1-cm diameter) obtained from 7-day-old culture. The inoculated medium was incubated at 30 ± 2°C for 15 days. The resulting fungal inocula were applied in pots experiment. *Trichoderma* inocula were adjusted, at 3.6×10^{8} propagules/gram, by using a haemocytometer slide. Then a mixture of each *Trichoderma* propagules was applied separately (El-Nagdi *et al.,* 2019).

2.3. Greenhouse experiments

Two pot experiments were carried out during two successive winter seasons of 2016/2017 and 2017/2018 in the greenhouse, Botany Department, National Research Center at Dokki, Cairo, Egypt.
The first experiment was conducted in the 1st season to examine the capability of *O. crenata* and *O. ramosa* to attack chamomile plants and their effects on chamomile growth parameters under greenhouse condition during 4 months after transplanting (MAT). Plastic pots (30-cm diameter), containing 5 kg of a sterilized mixture of loamy soil (48.4% sand, 41.1% silt, and 10.5% clay; pH 8.0) were arranged according to a completely randomized design on a bench under greenhouse conditions. The pots were separately artificially infested with each *Orobanche* sp. seeds at the rate of 5% (W/W) at 5 cm of the soil surface. The soil pots free of *Orobanche* sp. contamination were applied as the control. Then, four chamomile seedlings (30 days-old) were transplanted in each pot. Four pots were applied as replicates for each treatment as well as the control. The pots maintained at 25±5°C and the plants were irrigated and fertilized regularly. The numbers, fresh weight and dry weight of the emerged *Orobanche* were recorded at 1, 2, 3 and 4 MAT. The growth parameters of chamomile plants as plant height (cm), plant

fresh and dry weights (g), as well as fresh and dry weights of roots (g) were recorded during examined periods.

Second experiment was conducted in the second season to examine the efficacy of *Trichoderma* spp. for controlling *O. ramosa* in chamomile plants. Twenty plastic pots (30-cm diameter), containing 5 kg of a sterilized mixture of clay soil were arranged according to a completely randomized design on a bench under greenhouse conditions. The pots were separately artificially infested with *O. ramosa* seeds bank at the rate of 5% (W/W) at 5 cm of the soil surface. Then, *Trichoderma* spp. treatments were applied at a rate of 5% (w: w) before one week of transplanting and then all pots were watered. The soil infested with *O. ramosa* as well as soil free of *O. ramosa* contamination were applied as the controls. The treatments were as follows; *T. hamatum* + *O. ramosa*; *T. harzianum* + *O. ramosa*; *T. viride* + *O. ramosa*; *O. ramosa* alone and untreated control and then all pots were watered. Then, four chamomile seedlings (30 days-old) were transplanted in each pot. The pots maintained at 25±5°C and the plants were irrigated and fertilized regularly. Effects of *Trichoderma* spp. on numbers, fresh and dry weights of the emerged *Orobanche* were recorded at 1, 2, 3 and 4 MAT. The growth parameters of chamomile plants as plant height (cm), plant fresh weight (g), plant dry weight (g), root fresh and dry weights (g) were recorded over4 MAT.

2.4. Statistical analysis

The obtained data in two pot experiments were subjected to analysis of variance using Computer Statistical Package User Manual Version 3.03, Barkley Co., USA and mean values of treatments were compared by Duncan's multiple range test at P=0.05 level of significance (Snedecor and Cochran, 1999).

3. Results and Discussion

3.1. Sensitivity of chamomile plant to *O. crenata* and *O. ramosa* infestation

Capability of *O. crenata* and *O. ramosa* to attack chamomile plants was studied under greenhouse condition during 4 months after transplanting (MAT). The results revealed that chamomile plant is susceptible to *O. ramosa*, whereas it maintained complete resistance to *O. crenata* infestation (Table 1). Number of *O. ramosa* attachments developed during the examined period and constituted 10.8 per plant at the end of examined period (4 MAT). Also, fresh and dry weights of *O. ramose* plants were increased throughout the examined period and reached 28.1 and 4.53 g per plant, respectively, at 4 MAT. These results are in agreements with the obtained results by Habimana *et al.* (2014) and Punia (2014). They reported that *Orobanche* spp. could parasite on leguminous, oilseeds, solanaceous, cruciferous and medicinal plants. Meanwhile, Nawar and Sahab (2011) reported that O. *ramosa* attacks chamomile herbs, through their excreta (Habimana *et al.,* 2014).

3.2. Effect of *O. ramosa* infestation on chamomile growth

Growth parameters of infested and non-infested chamomile plants were measured during 4 MAT. Chamomile growth was affected negatively with *O. ramosa* infestation at all examined times (Table 2). Various growth parameters of infested plants exhibited significant decreases in comparison with non-infested plants. Height, fresh and dry weight of infested plants was in the range 14.8- 61.8 cm, 39.9-88.0 g and 4.5-17.7 g, respectively against 17.5-69.3 cm, 57.9-125.1 g and 6.7-27.6 g, respectively for non-infested plants. At the end of examined period (4 MAT), infestation caused great decreases in plant height (10.8%), fresh weight (29.7%) and dry weight (35.9%). Also, *O. ramosa* infestation produced a pronounced decrease in root growth, over the examined period. Since, values of root fresh and dry weights of infested plants ranged between 4.8-8.7 and 0.3-2.3 g, respectively against 5.0-12.9 and 0.9-3.4 g for non-infested plants, respectively. At the end of examined period (4 MAT), *O. ramose* reduced fresh and dry biomass of root with about 32%. Many investigators recorded tremendous damage during growth stages of host plants as affected with *O. ramosa* infestation (Hassan *et al.,* 2004; Al-Wakeel *et al.,* 2013; Habimana *et al.,* 2014). Due to their achlorophyllous nature, *Orobanche*is constrained to obtain their nutritional resources from the host (Westwood, 2000). The damage induced in the host plant by *Orobanche* parasitism differs for each *Orobanche*-host association (Fernández-Aparicio *et al.,* 2016). In some plants, the biomass loss equals to that accumulated by the parasite indicating that damage in the plant is directly attributed to the parasitic sink activity (Hibberd *et al.,* 1998). However, in other *Orobanche*-host associations the damage induced through negative effects on the plant photosynthetic machinery and hormonal balance (Mauromicale *et al.,* 2008).

Table 1: Growth of *O.ramosa* and *O.crenata* parasites on chamomile plant under greenhouse conditions (1st season)

MAT	Number of emerged spike		Fresh weight (g)		Dry weight (g)	
	O. ramosa	***O. crenata***	***O. ramosa***	***O. crenata***	***O. ramosa***	***O. crenata***
1	1.5[a]	0.0[b]	0.8[a]	0.0[b]	0.01[a]	0.0[b]
2	6.8[b]	0.0[b]	5.2[b]	0.0[b]	0.93[b]	0.0[b]
3	9.5[c]	0.0[b]	26.6[c]	0.0[b]	3.80[c]	0.0[b]
4	10.8[d]	0.0[b]	28.1[d]	0.0[b]	4.53[d]	0.0[b]

Values are given as means of three replicates. Means with the same letters in a row are not significantly different at P<0.05.

Table 2: Effect of *O. ramosa* infestation on chamomile growth during four months after transplanting (MAT) (1st season)

Plant growth												
Parameter	**Height (cm)**				**Fresh weight (g)**				**Dry weight (g)**			
MAT	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Infested plants	14.8[a]	32.0[b]	55.3[c]	61.8[d]	39.9[a]	43.1[b]	64.7[c]	88.0[d]	4.5[a]	14.0[b]	16.0[c]	17.7[d]
non-infested plants	17.5[a]	34.8[a]	57.5[a]	69.3[a]	57.9[a]	74.5[a]	103.4[a]	125.1[a]	6.7[a]	18.2[a]	20.5[a]	27.6[a]

Root growth								
Parameter	**Fresh weight (g)**				**Dry weight (g)**			
MAT	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Infested plants	4.8[a]	6.5[b]	8.5[c]	8.7[c]	0.3[a]	1.3[b]	1.8[c]	2.3[d]
non-infested plants	5.0[a]	8.2[a]	9.1[c]	12.9[d]	0.9[a]	1.8[b]	2.2[c]	3.4[d]

Values are given as means of three replicates. Means with the same letters in a column are not significantly differentat P<0.05.

3.3. Effect of *Trichoderma* spp. on controlling *O. ramosa* parasitism

The results showed that application of *Trichoderma* spp. capable to control *O. ramose* parasitism in chamomile plants by delaying attachment time and reducing the number and growth of emerged spikes (Table 3). The most effective *Trichoderma* spp. on *O. ramosa* parasitism was *T. hamatum* followed with *T. viride*. These two species completely protected chamomile plants against *Orobanche* infestation until 3 MAT. Also, at 4th month, *T. hamatum* and *T. viride* treatments caused great reduction in number of *O. ramosa* attachments (95.2% and 75.8%, respectively) and produced more than 90% reduction in fresh and dry weights of tubercles. Also, *T. harzianum* prevented *O. ramosa* attachment in chamomile plants for one month. During the last three times points (2-4 MAT) *T. harzianum* caused great decreases in number attachments (between 77.8-81.8 %), fresh weight (48.9-90.6%) and dry weight of tubercles (42.9-85.7%). In line of the obtained results, Boari and Vurro (2014) found that fungi can apply as bio-control agents against *O. ramosa*. Hence, *Trichoderma* extensively applied in agriculture worldwide for crop protection due to their antimicrobial activities in suppressing a broad range of phyto-pathogens (Harman, 2006).*T. harzianum* and *T. viride* produced potential herbicidal metabolites which could inhibit seeds germination or shoot and root growths of some herbs seedlings (Kuang *et al.,* 2016). Many investigators reported that *Trichoderma* spp. could play an important role in controlling *O. crenata* in pea and faba bean fields as a natural bio-herbicide (Abdel-Kader and El-Mougy, 2002; El-Dabaa and Abd-El-Khair, 2020). Also, Hyder *et al.* (2017) found that application of *Trichoderma* spp. reduced the number of *Orobanche* shoots. They suggested that presence of *Trichoderma* in the rhizosphere could colonize the plant roots, receives nutrients from root exudates and exchange for plant protection against biotic and abiotic stresses. *Trichoderma* secretes the low molecular weight compounds, peptides and proteins as well as it have antagonistic mechanisms include mycoparasitism, antibiotic production and competition for nutrients. Moreover, *Trichoderma* can exert an indirect control against pathogens through the induced systemic response (ISR) in plant cells that enhanced the plant defense (Benítez *et al.,* 2004; Harman, 2006; Hermosa *et al.,* 2013). Moreover, Barghouthi and Salman (2010) reported that bacteria may control parasitic weeds by interrupting signals required for radical elongation, haustorium formation, rhizotropism or attachment.

Table 3: Effects of *Trichoderma* spp. on growth of *O. ramosa* infested on chamomile plant during four months after transplanting (MAT)

Parameters	**Number of emerged spike**				**Fresh weight (g)**				**Dry weight (g)**			
MAT	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
T. hamatum* + *O. ramosa	0.0^{b}	0.0^{b}	0.0^{b}	0.8^{a}	0.0^{b}	0.0^{b}	0.0^{b}	0.1^{a}	0.0^{b}	0.0^{b}	0. 0^{b}	0.1^{a}
T. viride* + *O. ramosa	0.0^{b}	0.0^{b}	0.0^{b}	4.0^{a}	0.0^{b}	0.0^{b}	0.0^{b}	2.2^{a}	0.0^{b}	0.0^{b}	0. 0^{b}	0.4^{a}
T.harzianum* +*O. ramosa	0.0^{c}	1.0^{b}	1.0^{b}	3.0^{a}	0.0^{c}	2.3^{b}	2.6^{b}	3.4^{a}	0.0^{b}	0.4^{a}	0.7^{a}	0.9^{a}
***O. ramosa* alone**	2.3^{d}	4.5^{c}	7.5^{b}	16.5^{a}	0.5^{d}	4.5^{c}	18.9^{b}	36.3^{a}	0.1^{d}	0.7^{c}	4.9^{b}	5.3^{a}

Values are given as means of three replicates. Means with the same letters in a column are not significantly different at P<0.05.

3.4. Effect of *Trichoderma* spp. on growth of chamomile plants infested with *O. ramosa* parasite

The effect of *Trichoderma* spp. on growth of chamomile plants artificially infested with *O. ramosa* in pots are presented in Table 4. Infestation of chamomile plants with *O. ramosa* tended to cause significant reductions in various growth parameters of chamomile during 4 MAT (Table 4). Height of infested plants ranged between 18.5-58.8 cm corresponded with 21.5-74.5 cm for *Orobanche* non-infested plants. Adding *Trichoderma* fungi in the soil of *O. ramosa* infested plants improved plant height of chamomile plant, in most cases. *T. viride* produced the most enhancement effects on plant height (between 8.1-40.7%), compared with *Orobanche* infested plants over the examined times. Although, application of *T. harzianum* did not produce any significant effects on plant height during the last two times points (3 and 4 MAT). As shown in Table 4, *Orobanche* infestation caused great reduction in chamomile biomass, as compared with non-infested plants. The reduction in fresh biomass over the examined periods was in the range 13.9-39.4% accompanied by 7.9-64.9% reduction in dry biomass. Presence of *Trichoderma* fungi in the pots of *O. ramose* infested plants induced great increase in fresh and dry weights of chamomile plants when compared with those of infested plants alone (Table 4). At 2nd MAT, *Trichoderma* spp. increased fresh plant biomass between 44.5-100%, as compared with infested plants alone and between 13.3-59.9%, as compared with control (non-infested plants). Such increase was accompanied by increase in plant dry matter constituted between 62.1-79.3%, relative to infested plants and 49.2-65.1%, relative to healthy non-infested plants.

Fungi treatments continued to exert great enhancingin plant biomass of *Orobanche* infested plants over the examined period. At 4th MAT, increases in fresh and dry weight of *Trichoderma* treated plants were in the range 59-90% and 67.6-112.2%, respectively compared with infested plants. As shown in Table 4, *Trichoderma spp.* varied in their enhancing effects on fresh and dry weights of plants over the examined period. At 1st MAT, *T. hamatum* produced the highest values of fresh and dry weight of chamomile plants (26.8 and 3.8 g, respectively) as compare with infested plant alone (24.1 and 3.0 g, respectively). At 2nd MAT, *T. harzianum* produced the highest increase in fresh and dry weights of infested plants (100 and 79.4%, respectively). Meanwhile, at the last two times points, *T. viride* was superior in enhancing fresh and dry plant biomass. It produced increases reached 85.1 and 91.2%, respectively, relative to infested plants as well as 27.6 and 6.6% relative to non-infested plants. By the end of examined period, *T. viride* continued to produce the maximum increases in plant fresh and dry weights as compared with infested plants (89.7 and 112.1%). The enhancement effects of different *Trichoderma* spp. on plant growth were previously observed in parsley (Barroso *et al.,* 2019)**,** coriander (Gebarowska *et al.,* 2019) and *Passifloracaerulea* L. (Şesan *et al.,* 2020). Moreover, an enhancement effect of *Trichoderma* in growth of *Orobanche* infested plants was previously observed by Hyder *et al.*

(2017) and El-Dabaa and Abd-El-Khair (2020). Improving soil fertility by using beneficial microorganisms appeared to decrease *Orobanche* infestation and its suppressive effects on host growth (Elabaied *et al*.,2018).With accordance with our results, Khan and Parveen (2018) observed the superiority of *T. viride* in enhancing the growth and yield of coriander infected with *Protomyces macrosporus*.

Table 4: Effect of *Trichoderma* spp. on growth of chamomile plants infested with *O.ramosa* parasite during four months after transplanting (MAT)

Plant growth												
Parameters	**Height (cm)**				**Fresh weight (g)**				**Dry weight (g)**			
MAT	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
T. hamatum* *+O. ramosa	20.0^{d}	32.5^{c}	48.3^{b}	64.5^{a}	26.8^{d}	50.9^{c}	64.3^{b}	78.4^{a}	3.8^{d}	9.4^{c}	12.2^{b}	14.8^{a}
T. viride* *+O. ramosa	22.5^{d}	33.5^{c}	58.1^{b}	69.1^{a}	21.1^{d}	50.3^{c}	76.8^{b}	93.5^{a}	3.0^{d}	9.5^{c}	13.0^{b}	15.7^{a}
T. harzianum* *+O. ramosa	20.3^{d}	27.3^{c}	40.3^{b}	59.0^{a}	22.3^{b}	71.0^{a}	73.6^{a}	79.6^{a}	3.4^{b}	10.4^{a}	12.1^{a}	12.4^{a}
***O. ramosa* Only**	18.5^{d}	31.0^{c}	41.3^{b}	58.8^{a}	24.1^{c}	34.8^{b}	41.5^{b}	49.3^{a}	3.0^{c}	5.8^{b}	6.8^{b}	7.4^{a}
Control (untreated plants)	21.5^{d}	29.8^{c}	55.8^{b}	74.5^{a}	28.0^{d}	44.4^{c}	60.2^{b}	81.4^{a}	4.5^{d}	6.3^{c}	12.2^{b}	21.1^{a}

Root growth								
Parameters	**Fresh weight (g)**				**Dry weight (g)**			
MAT	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
T. hamatum+O. ramosa	4.6^{d}	8.3^{c}	12.2^{b}	15.4^{a}	0.7^{d}	1.3^{c}	2.2^{b}	2.7^{a}
T. viride+O. ramosa	4.2^{d}	13.0^{c}	18.7^{b}	24.4^{a}	0.7^{d}	1.9^{c}	2.8^{b}	3.7^{a}
T. harzianum+O. ramosa	5.4^{c}	15.2^{b}	15.5^{b}	20.5^{a}	0.8^{c}	2.4^{a}	2.4^{b}	2.7^{b}
***O. ramose* alone**	4.0^{b}	6.1^{c}	9.2^{c}	9.9^{d}	0.4^{b}	0.7^{c}	1.0^{d}	1.4^{c}
Control (untreated plants)	4.1^{d}	5.9^{c}	9.9^{b}	12.5^{a}	0.4^{c}	0.7^{b}	1.4^{a}	1.6^{a}

Values are given as means of three replicates. Means with the same letters in a column are not significantly differentat $P<0.05$.

In conclusion, the obtained results revealed that adding of *Trichoderma* spp.to the soil successfully reduced both infection and intensity of attack with *O. ramosa* on chamomile and enhanced the host growth. So, application of *Trichoderma* fungi may be provide ecofriendly strategy for crop disease management and can be a useful tool in sustainable modern agricultural practices and economically feasible.

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