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Management of root holoparasite *Aeginetia pedunculata* of (Orobanchaceae), causing wilt of sugarcane by trap and catch crops

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A B S T R A C T

Sugarcane wilt caused by *Aeginetia pedunculata* (Roxb.) Wall. (Orobanchaceae) is endemic in the command area of Plassey Sugar Mill in the districts of Nadia and Murshidabad in West Bengal. It spreads through water-and soil-borne seeds. With the objective of developing location-specific integrated management schedule of the parasite, crops, other than sugarcane, grown in the target area were evaluated for their potential as trap and catch crops of the parasite. Twelve crops, selected on the basis of germination induction test of *A. pedunculata* seeds on root exudates as compared to that of three germination inducing chemicals (*viz.;* NaOCl, TTC (2,3,5-triphenyl tetrazolium chloride) and GR24-a strigol analogue) and growth of the parasite on excised root pieces of prevalent crop cultivars including sugarcane, were evaluated in *A. pedunculata* infested sugarcane field for their (crops) efficacy to deplete or recharge the parasite seeds in soil. Rice (*Oryza sativa* L.), maize (*Zea mays* L.), pearl millet (*Pennisetum typhoides* Staph. & Hubbard.), italian millet (*Setaria italica* (L.) Breauv.), black gram (*Phaseolus mungo* L.), dhaincha (*Sesbania aculeata* (Willd.) Pers.), sesame (*Sesamum indicum* L.), jute (*Corchorus olitorius* L.), pigeon pea (*Cajanus cajan* (L.) Millsp.), and groundnut (*Arachis hypogaea* L.) were found effective as trap crops for their ability to germinate the seed of *A. pedunculata*. Sorghum (*Sorghum bicolor* (L.) Moench) was identified as a catch crop for its ability to support further growth and development of *A. pedunculata* up to flowering.

Keywords : Holoparasitic angiosperm, Orobanchaceae, *Aeginetia pedunculata,* integrated management, trap crop, catch crop, seed viability, germination, soil seed bank, Sugarcane (*Saccharum* spp.)

Introduction

Sugarcane wilt caused by the root holoparasite *Aeginetia pedunculata* (Roxb.) Wall. (Orobanchaceae) is the main problem of sugarcane in the command area of Plassey Sugar Mill, covering nearly 45 km^2 in Nadia and Murshidabad districts of West Bengal (Ray & Dasgupta, 2003; 2006a). Crop loss involves 58% reduction of sucrose in juice, and 36% (1.89 t/ha) reduction of commercial cane sugar per ha (Ray & Dasgupta, 2006b). Management of the parasite is difficult because hand weeding and spraying of herbicides (such as 2,4-D Na; at 2 kg ai/ha) are effective in destroying the aerial part of the parasite, while underground rhizome (scape) remains intact contributing to the existing seed bank in soil leading to fresh infection after germination. One of the components of integrated management of the parasite could be introduction of suitable trap and catch crops under sugarcane based cropping system / rotation. While both trap and catch crops allow the parasite seeds to germinate, the former do not get parasitized but the later are parasitized by the parasite after germination (Kleifield *et al.* 1994).

While natural hosts of *A. pedunculata* is limited to sugarcane, sorghum and a few wild grasses in India (Ray & Dasgupta 2009) *A. indica*, another root holoparasite of sugarcane, parasitize dryland rice (*Oryza saliva* L.), maize (*Zea mays* L.), italian millet (*Setaria italica* Breauu), Japanese ginger (*Zingiber mioga* Rosc.) and many monocot and dicot weeds in Japan, Philippines and India (Kusano 1903; Kusano 1908; Subramaniam 1936; Espino 1947). These short duration crops can be grown in rotation with sugarcane as catch crop but trap crops of *A. pedunculata* are yet to be ascertained. For management of other major root parasites such as *Orobanche* and *Striga* some trap and catch crops have been reported. Winter crops like flax (*Linum usitatissimum* L.), clover (*Trifolium alexandrinum* L.) and vetch (*Vicia sativa* L.) and summer crops like mung bean (*Phaseolus aureus* Roxb.) and sorghum (*Sorghum bicolor* (L.) Moench) grown as trap and/or catch crop decreased infestation of *Orobanche aegyptiaca* Pers. and increased vigour and yield in tomato (Kleifield *et al.* 1994). Intercropping with a spreading type of cowpea (*Vigna unguiculata*) (Carsky *et al.* 1994) or groundnut (Gworgwor 2000) controlled *Striga hermonthica* (Del.) Benth. on sorghum. Soybean (cv. TG × 1740-7F) was effective in both germinating the seed *in vitro* and reducing *S. hermonthica* infestation in maize with concomitant increase in maize yield up to 90% (Carsky *et al.* 2000). Fodder legumes like Silverleaf, *Desmodium uncinatum* (Jacq.) DC. and Greenleaf, *D. intortum* (Mill.) Urb. (Fabaceae) reduced the infection of maize by giant witchweed (*S. hermonthica*) by allelopathy (Khan *et al.* 2002). Root exudates of cotton (*Gossypium hirsutum* L. and *G. barbadense* L.) germinated the seed of *S. hermonthica* and reported to be used as a trap crop (Botanga *et al.* 2003).

Present investigation was aimed at identifying potential trap or catch crops which can be grown in rotation with sugarcane in order to reduce the parasite seed load in soil under the ecosystem under study.

Materials and Methods

Viability and germination of A. pedunculata seed under non-parasitic condition

This experiment was conducted to identify suitable chemicals and technique for inducing germination of *A. pedunculata* seeds in absence of host for viability study *in vitro*. Mature and dry capsules of *A. pedunculata* were collected from infected sugarcane fields in different farms of Plassey Sugar Mill, Plassey, Nadia, West Bengal, India (PSM) each year during November 2001 to 2006. The seeds were air dried, packed in double polythene packets, labelled and stored under ambient conditions in the laboratory of Sugarcane Research Station, Bethuadahari, Nadia, West Bengal, India. Effect of different chemicals on germination of parasite seed was tested in petri dishes (9 cm dia) using moistened (with respective chemicals) filter paper discs. Sterilized distilled water soaked filter paper was maintained as control. Each treatment (chemical) was replicated four times. In each petri-dish 1000 seeds of the parasite were placed and were incubated in BOD at 35°C for 7 days. Percentages of seed germination, seed viability and contamination were determined using a microscope (100×), by counting 100 seeds randomly per plate on each of the four replicates. Mean values and standard deviations were calculated. The treatments were:

T_1 = NaOCl (1%) for 5 min and washed thoroughly with sterile distilled water (French & Sherman 1976, Kato & Hisano 1983).

T_2 = TTC (1%) for 72 h at 35°C then NaOCl (4%) for 5 min to bleach the dark seed coat. Red or pink embryos were considered viable and those not were non-viable or dead (Lopez-Granados & Garcia-Torres 1999).

T_3 = GR 24 (a strigol analogue) (0.034mM) for 7 days. Stock solution (100ppm) was prepared by dissolving the same in a few drops of acetone and adding sterile distilled water to the desired concentration (5-20 ppm) and stored at 0°C (van Hezewijk *et al.* 1993).

T_4 = Sugarcane root exudates collected by dipping the root in 100 ml sterile distilled water for 7 days

T_5 = Control (sterile distilled water)

Germination of A. pedunculata seed with root exudates or excised root pieces

To study the effects of different crops on seed germination (*A. pedunculata*) *in vitro*, a similar experiment was conducted using root exudates of the crops. Root exudates were collected by dipping the roots of seedlings in 100 ml sterile distilled water for 7 days in a test tube plugged with non-absorbent cotton. For determining the behaviour (germination) of the parasite seed in contact with crop roots, filter paper were moistened with sterile distilled water and spread with 2 cm excised root tips of the crop seedlings. Approximately, 1000 seeds of *A. pedunculata* were placed on the moist filter paper and were incubated at 35°C for 7 days in a BOD incubator. Percentages of seed germination was determined using a microscope (100×), by counting 100 seeds randomly per plate of four replications. Mean values and standard deviations (P = 0.05) were calculated.

Growth and development of A. pedunculata on living roots of crops

To evaluate crops for their (living roots) ability to induce germination (seed) and growth of the parasite, the experiment was conducted in polythene packets (10 cm × 8 cm). Each packet was filled with a sand soil (50:50) mixture, mixed with 0.5 g seeds of *A. pedunculata*. The packets were perforated at the bottom for drainage of excess water. Seeds of different crops including sugarcane (single node cuttings) was sown / planted in the packets, moistened and kept under ambient room temperature conditions (mean temperatures during the period of experiment were: Max 32°C, Min 22°C). After germination of host plant the packets were planted in an experimental plot in rows 90 cm apart with spacing of 60 cm between plants (polythene packets) at the Sugarcane Research Station farm. The experiment was laid out in RBD with three replications. After 90 days, roots were washed carefully to observe the growth and flowering of *A. pedunculata*, *in vivo* (Juliano 1935). The crops evaluated were sugarcane (*Saccharum* sp. complex hybrid, cv BO 91), rice (*Oryza sativa* L., cv MTU 7029), sorghum (*Sorghum bicolor* (L.) Moench., hybrid for forage), pearl millet (*bajra*) (*Pennisetum typhoides* Staph. & Hubbard., cv local), italian millet (*kaon*) (*Setaria italica* (L.) Breauv., cv local), maize (*Zea mays* L., hybrid), black gram (*Phaseolus mungo* L., cv Kalindi), *dhaincha* (*Sesbania aculeata* (Willd.) Pers., cv local), sesame (*Sesamum indicum* L., cv Rama), jute (*Corchorus olitorius* L.), pigeon pea (*Cajanus cajan* (L.) Millsp., cv local) and groundnut (*Arachis hypogaea* L., cv local) along with a non-crop control. Mean values and standard deviations were calculated (P = 0.05).

Effect of different crop rotations on A. pedunculata seed bank in soil

Different cropping rotations including sugarcane (new plant or ratoon of cv. BO 91) were evaluated in the field (Plot no. 10, Manikdihi Farm of Plassey Sugar Mill) for their efficacy to reduce or recharge *A. pedunculata* seed bank in soil, during 2003. The soil was severely infested with *A. pedunculata*. After harvesting of sugarcane in December 2002, the plot was subdivided into 28 plots, which were subjected to following 7 treatments in RBD each with 3 replications.

T_1 = Sugarcane cv. BO 91, planted in February 2003, harvested in December 2003

T_2 = Sugarcane ratoon maintained in December 2002, harvested in November 2003

T_3 = Boro (winter) rice cv. IR 36 transplanted in January 2003, harvested in May 2003

T_4 = Forage sorghum cv. hybrid, sown in March 2003, harvested in June 2003

T_5 = Jute cv. Naveen (JRO 524), sown in April 2003, harvested in August 2003

T_6 = Sesame cv. Rama, sown in March 2003, harvested in July 2003

T_7 = Fallow from December 2002 to November 2003

From each plot 1kg soil sample composited from five soil samples was collected from 0-15 cm depth during December 2002 and November 2003, with the help of soil augur, air-dried and thoroughly mixed. Each soil sample was mixed with 10 l of water, stirred well and sieved through a series of 44, 60, 85 and 100 mesh sieves. The supernatants were observed under microscope and *A. pedunculata* seeds were counted (Krishnamurthy & Chandwani 1975). The increase or decrease in the number of seeds was calculated to evaluate the recharge or depletion of seed load of the parasite in soil. The data were analysed for ANOVA (P = 0.05) to work out critical differences between treatments.

Results and Discussion

Viability and germination of A. pedunculata seed under non-parasitic condition

Matured seeds of *A. pedunculata* are roughly oval, slightly elongated at the micropylar end (ovoid pyriform), very minute, 0.3 mm long and 0.2 mm wide. Thousand seed weight (dry) was 0.138 g. Seeds were brown, highly pitted and float in water for more than 7 days. Earlier attempt to germinate *A. pedunculata* seeds sourced from the grass *Themeda triandra* Forsk grown on the slopes of Wynad Ghats (Kerala, India) were failed in spite of 24-48 h pre-treatment in dark and treatment with either 5 ml CEPA (2-chloroethylphospheric acid) (a ethylene releasing agent) at 0.1 or 1.0 or 10.0 or 100 mg/l, or plant pieces of *T. triandra* or root exudates of *T. triandra* (by dipping the roots 15 day old seedlings of *T. triandra* in 50 ml water for 7 days) in moist filter paper (Niranjana 1994). However, in the present investigation, seeds of *A. pedunculata* showed significantly higher germination over control (13.6%) when treated with NaOCl (93.3%), TTC (87.6%), GR24 (84.4%) and sugarcane root exudates (30.3%) (Table 1). Germination of *A. pedunculata* seed comprises only the growth of parenchyma tissue from the micropylar end, which lacks true plumule or radicle. Therefore seeds were considered as germinated when the length of growing tissue exceeds its width. Fungal contamination was observed in Petri dishes with sugarcane root exudates (45.8%), sterile distilled water (44.4%) and GR24 (13.8%) but no contamination was observed with NaOCl or TTC.

Table 1.

Effect of chemical treatments on seed viability and germination

Treatments	Viability (%)	Germination (%)	Contamination (%)
NaOCl		93.3	0.0
TTC	94.2	87.6	0.0
GR24		84.4	13.8
Sugarcane root exudates		30.3	45.8
Sterile distilled water		13.6	44.4
CD (*P=0.05*)		6.45	8.03
CV (%)		28.9	100.1

Treatment with TTC clearly demonstrated high (94.2%) seed viability by colouring the endosperm. At the same time TTC supported higher seed germination of *A. pedunculata*, which was not reported earlier for any parasitic plant. On the other hand, a few germinating seeds did not show any colouration. NaOCl sometimes bleach the seed, GR24 cannot indicate the viability of seed and sugarcane root exudates gives erratic result due to unknown level of actual ingredients. Based on the observations TTC was identified as most suitable chemical for testing viability, dormancy and germination of *A. pedunculata* seed without having any need for pre-sterilization.

Seeds of parasitic plants usually do not germinate only by imbibitions. But seeds of *A. pedunculata* germinated (13%) without any chemical stimulation at 35°C in BOD. This indicated breaking of dormancy with the influence of environmental conditions. This phenomenon was never observed in cases of the seeds of *Striga* or *Orobanche* and due to this reason the seed bank of *A. pedunculata* rapidly diminish in natural soil and the spread of the parasite is restricted to specified areas.

Germination of A. pedunculata seed with root exudates or excised root pieces

Root exudates of sugarcane (47.3%), black gram (46.3%), sorghum (40.7%), maize (33.7%), groundnut (30.3%), rice (29.0%), italian millet (*kaon*) (27.6%) and pigeon pea (26.8%) significantly increased germination of *A. pedunculata* seed over control (19.3%) (Table 2). On the other hand, excised root pieces of sorghum (62.8%), rice (61.8%), sugarcane (61.1%), black gram (55.6%), pearl millet (*bajra*) (55.1%), maize (42.6%), groundnut (40.6%), *dhaincha* (31.2%), jute (29.4%) and pigeon pea (28.2%) significantly induced higher germination of *A. pedunculata* seed over control (19.3%) (Table 2). Root exudates (18.1%) and excised root pieces (16.9%) of sesame however were not effective in inducing germination. Contrary to the earlier reports plants belong to Leguminosae (black gram, groundnut and pigeon pea) also induced germination of *A. pedunculata* seed, apart from by members of Poaceae alone.

Growth and development of A. pedunculata on living roots of crops

Tubercles of *A. pedunculata* were observed on roots of sugarcane (4.7), sorghum (2.3), maize (2.0) and pearl millet (*bajra*) (0.7). Tubercles were associated with soft jelly like rhizome or scape in sugarcane (2.0), maize (1.3) and sorghum (1.0). Only sugarcane (5.7) (Fig. 3) and sorghum (0.7) developed flower buds above the ground. First flowering of *A. pedunculata* took 124 days in sugarcane, by which time other crops were already harvested.

Depletion of A. pedunculata seed in soil under different crop rotation sequences

Under uniform initial seed load (36500 to 45600 seeds per m^2) of *A. pedunculata* in the soil, planting of sugarcane nearly doubled (97.5%) the seed load in one cropping season. At the same time, maintenance of sugarcane ratoon had increased the number of seed in soil more than three times (369.2%) than the initial level. Contrary to this, crop rotation with rice (72.3%), jute (70.9%), sesame (60.7%), sorghum (59.0%) and fallow (52.5%) had reduced the seed load by more than half of the initial level. The differences however among these treatments were not statistically significant. However, among the four crops tested, rice and jute were better in reducing the parasite seed load in soil (Table 4). These results corroborate with the results of seed germination tests *in vitro* with root exudates or excised root pieces.

Sorghum, which actually hosts the parasite in the field, can be grown as a catch crop. Other crops such as rice, jute, sesame, dhaincha, black gram, maize, groundnut and pigeon pea can be grown as trap crops in rotation with sugarcane as a component of a location specific integrated management programme of *A. pedunculata* on sugarcane in the *A. pedunculata* endemic areas. Also, there are other considerations for selection of the crops in rotation such as compatibility with the existing cropping system, economic viability and farmers' preference.

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Table 2.
Effect of root exudates and excised root pieces of different crops on the germination of seed of *A. pedunculata* on filter paper

Treatment crop	Species	Cultivar	Germination (%) with	
			Root exudates	Excised root piece
Sugarcane	*Saccharum officinarum* L.	BO 91	47.3	61.1
Rice	*Oryza sativa* L.	MTU 7029	29.0	61.8
Sorghum	*Sorghum bicolor* (L.) Moench.	Hybrid	40.7	62.8
Pearl millet	*Pennisetum typhoides* Staph. & Hubbard.	Local	20.2	55.1
Italian millet	*Setaria italica* (L.) Breauv.	Local	27.6	28.7
Maize	*Zea mays* L.	Hybrid	33.7	42.6
Black gram	*Phaseolus mungo* L.	Kalindi	46.3	55.6
Dhaincha	*Sesbania aculeata* (Willd.) Pers.	Local	22.7	31.2
Sesame	*Sesamum indicum* L.	Rama	18.1	16.9
Jute	*Corchorus olitorius* L.	Naveen (JRO 527)	25.2	29.4
Pigeon pea	*Cajanus cajan* (L.) Millsp.	Local	26.8	28.2
Groundnut	*Arachis hypogaea* L.	Local	30.3	40.6
Control			19.3	19.3
CD (*P=0.05*)			6.5	7.2
CV (%)			22.6	17.9

Table 3.
Growth and development of *A. pedunculata* on living root of crops

Treatment crop	Cultivar	Tubercle formed	Rhizome developed	Flower emerged
Sugarcane	BO 91	4.7 (2.24)	2.0 (1.55)	5.7 (2.46)
Rice	MTU 7029	1.0 (1.17)	0.0 (0.70)	0.0 (0.70)
Sorghum	Hybrid	2.3 (1.65)	1.0 (1.17)	0.7 (1.05)
Pearl millet (*Bajra*)	Local	0.7 (1.05)	0.0 (0.70)	0.0 (0.70)
Italian millet (*Kaon*)	Local	0.0 (0.70)	0.0 (0.70)	0.0 (0.70)
Maize	Hybrid	2.0 (1.55)	1.3 (1.34)	0.0 (0.70)
Black gram	Kalindi	0.0 (0.70)	0.0(0.70)	0.0 (0.70)
SE (±)		(0.185)	(0.137)	(0.097)
CD (P=0.05)		(1.489)	(0.405)	(0.342)

Figures in the parenthesis are square root transformed values

Table 4.
Effect of crop on *A. pedunculata* seed load in soil

Treatments (After harvesting of infected sugarcane plant crop)	*A. pedunculata* seeds in soil ('000 /m^2)		Change (%)
	Before planting/ sowing	After harvesting	
Sugarcane (Plant)	44.6	88.1	+97.5
Sugarcane (Ratoon)	44.8	210.2	+369.2
Rice	36.5	10.1	-72.3
Sorghum	41.5	17.0	-59 .0
Jute	44.0	12.8	-70.9
Sesame	44.8	17.6	-60.7
Fallow	45.6	21.6	-52.5
CD (P = 0.05)	NS	10.8	
CV (%)	86.3	46.0	

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