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Effects of applied leaf biomass of *Parthenium hysterophorus*, *Cassia obtusifolia* and *Achyranthes aspera* on seed germination and seedling growth of wheat and pea

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ABSTRACT

We investigated the effects of leaf biomass of *Parthenium hysterophorus* L., *Cassia obtusifolia* L. and *Achyranthes aspera* L., weeds on seed germination and seedling growth of wheat and pea and changes in the soil organic carbon. Soils were amended with leaf biomass (1, 2 and 5 g/Kg soil) of these weeds and the mixtures of leaf biomass of *P. hysterophorus* (5 g/Kg soil) with *C. obtusifolia* or *A. aspera* (5 g/Kg soil). The leaf biomass of weeds significantly improved the soil organic C pool and influenced the crop growth. However, their impact was crop specific and depended on doses and quality of leaf biomass (weed type). The mixtures of *P. hysterophorus* had variable effects (additive, antagonistic and synergistic) on the crop growth, thus, weed biomass may have potential in weed management.

Key words: *Achyranthes aspera* L., *Cassia obtusifolia* L., Crop growth, leaf-biomass, *Parthenium hysterophorus* L., pea (*Pisum sativum* L.), seed germination, weed, wheat (*Triticum aestivum* L.)

INTRODUCTION

Weeds adversely affect the growth of crop plants through competition for growth resources, altering the soil microbiological processes and release of allelochemicals. The invasive plant species have the early successional traits e.g. fast colonization, fast reproduction, or high competitive/allelopathic ability, to invade disturbed sites (27,38). The allelopathic activity of these weeds alters the soil environment and accelerates their fast spread by eliminating the native species (5). The biomass of weed leaves after decomposition changes the soil characteristics (13) and is relatively tender than that of stems. About half of leaf plant biomass is composed of carbon (37), hence, leaves notably contribute to the soil organic carbon. Incomplete removal of weed-biomass from the fields, adversely affects the succeeding crop yield (8) and biodiversity in ecosystem (24,28). During the decomposition, weeds release water soluble allelochemicals in bioactive concentrations, which may directly affect the plant growth (1,12), nutrients availability (2) and other soil characteristics (39,41). Over long time, such changes in soil properties may greatly affect the species composition and crop growth performance in agroecosystems.

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Parthenium hysterophorus L. (congress grass) has invaded and spread throughout India (16, 25). It is a short-lived exotic weed with very high reproduction rate (11). It completes 2-3 generations per year and has enormous seed bank (~200,000 seeds/m^2) in abandoned fields in India (22). *Cassia obtusifolia* L. (sicklepod) is aggressive invasive weed (15,21) and has high germination and reproduction rate (3). *Achyranthes aspera* L. (prickly chaff-flower) is a common weed on road sides and fallow lands and is used as folk medicine (17). Among the exotic weeds, *P. hysterophorus* is most aggressive invasive weed in India (since its entry in 1951). In North India *P. hysterophorus*, *C. obtusifolia* and *A. aspera* are common weeds in crop fields, after rainy season (19). The allelopathic research has been done on *P. hysterophorus* (4,16,35,36), but little is known about *C. obtusifolia* and *A. aspera*. The impact of only *P. hysterophorus* has been studied on soil, but not in combination with other weeds. Such study is important, because weeds often coexist in fields and may release various growth retardants (6).

This study investigated the impact of leaf-biomass of *P. hysterophorus*, *C. obtusifolia* and *A. aspera,* on the growth of wheat and pea. The main objectives were: (i) to investigate growth and seed germination of wheat and pea in soils amended with varying amounts of leaf-biomass of *P. hysterophorus*, *C. obtusifolia* and *A. aspera* separately or in mixtures and (ii) to determine changes in the organic carbon content in soils amended with weeds' leaf-biomass.

MATERIALS AND METHODS

Fresh leaves of mature plants (reproductive phase) of *Parthenium hysterophorus*, *Cassia obtusifolia* and *Achyranthes aspera* (hereafter referred to as Ph, Co and Aa respectively) were collected from the weed-infested sites in Bulandshahr (28º 24´ N lat. and 77º 51´ E long), India, in end of October 2006. The leaves were oven-dried (60 ºC for 48 h), powdered and stored in polythene bags at room temperature for use in soil experiments.

Pot Culture

Powdered leaf of weeds (Ph, Co, Aa) was added at 1, 2, 5 g per Kg soil on December 24, 2006. One Kg soil was put in each polythene bag (perforated bottom) for pot culture investigations. The soil was collected from crop fields [(pH 7.95 ± 0.05, organic C 0.92 ± 0.03 %, total N 0.05 ± 0.003 %, available P 0.041 ± 0.002 mg/g, exchangeable K 0.69 ± 0.04 mg/g and Ca 0.99 ± 0.02 mg/g (18)]. The leaf-biomass doses were based on the weed-leaf biomass present in crop fields (18). To study the impact of Ph in combination with other weeds, 10 g Ph leaf-biomass alone or 5 g in mixture with 5 g Co or Aa were added separately to 1 Kg soil. Unamended soil served as control. Nine replicates (three for wheat, three for pea and three without any crop) were prepared for each applied weed-leaf biomass treatment. The treatments were labeled as Ph1, Ph2, Ph5, Co1, Co2, Co5, Aa1, Aa2, Aa5, Ph10, Ph5Co5, Ph5Aa5 and control. The digits suffixed to species codes (Ph, Co, Aa) indicated the amount (g) of applied leaf-biomass of a species. Bags were watered to field capacity daily throughout the experiment, and the unwanted, emerged plants were eliminated periodically.

The treatment consisted of 3 factors: (i). Donor weeds 3 (*Parthenium hysterophorus* L., *Cassia obtusifolia*, L. and *Achyranthes aspera* L.) (ii). Applied Doses 3 (1, 2, 5 g/Kg soil) and (iii). Recipient crops 2 [wheat (*Triticum aestivum* L. var. PBW 373) and pea (*Pisum sativum* L. var. Arkil)]. Ten days after watering and weeding (January 2007), 10 seeds of wheat and 6 seeds of pea were sown separately in the amended soils in triplicate. Percent germination was recorded until no further seeds germinated. For growth studies, three healthy individuals were allowed to grow and the rest of the germinated plants were eliminated. Periodically the following growth parameters were measured: shoot length, basal diameter and number of leaves. The first measurement was taken for both crops 20 days after sowing (DAS), followed by measurements taken at 30, 40, 50, 70, 90 and 110 DAS for wheat, and at 27, 34, 41, 55, 69 and 83 DAS for pea. Total shoot biomass of crop plants was harvested at 83 DAS on 28 March 2007 for pea and 110 DAS on 24 April 2007 for wheat and oven-dried at 80 ºC for 48 h. The experiments were carried out under shaded-house conditions with natural light supply. During the period of the crop growth study, the recorded mean maximum temperature was 25.1 ºC with a minimum of 12.2 ºC and the mean maximum relative humidity was 72 % with a minimum of 45 %.

Soil: One month after soil amendments (January 24, 2007), soil samples were collected, air-dried and analyzed for soil organic C using the rapid titration method (40).

Statistical Analysis:

The statistical analysis was done using SPSS 16.0 (SPSS Inc., Illinois) and Genstat 11.0 (VSN International Ltd.). To differentiate weed leaf-biomass interactions of binary mixtures a method based on multiplicative survival model was used (9). Colby's formula ($E = A*B/100$) was used to calculate the expected growth E in an additive response based on the growth values obtained for the two single treatment means (A and B in terms of biomass of crop plants as % of control). A paired t-test compared the expected growth value (E) with the observed growth value for the binary leaf-biomass mixtures (A+B) and determined if responses were significant synergistic or antagonistic.

RESULTS

The leaf-biomass amendments of the three weeds tested alone did not show any significant effect on the germination of wheat and pea seeds with one exception (Fig. 1). The germination percent of both crops slightly increased in Co- and Ph-amended soils, albeit insignificant. Just in Aa-amended soils, pea seed germination declined significantly by 50 % at the highest leaf-biomass content (5 g/Kg soil). In soils amended with higher leaf-biomass (10 g/Kg soil) seed germination showed variable trends. *P. hysterophorus* alone (Ph10) stimulated both wheat (4%) and pea (50%) seed germination. However, in soils amended with its binary mixtures (Ph5 Co5 and Ph5 Aa5), inhibition of seed germination was recorded for both wheat (25% in Ph5 Co5 and 7% in Ph5 Aa5) and pea (75% in Ph5 Co5 and 62% in Ph5 Aa5) crops.

The wheat and pea plants showed variable growth trends in soils amended with the different weed leaf-biomass. The shoot length of wheat plants increased with time

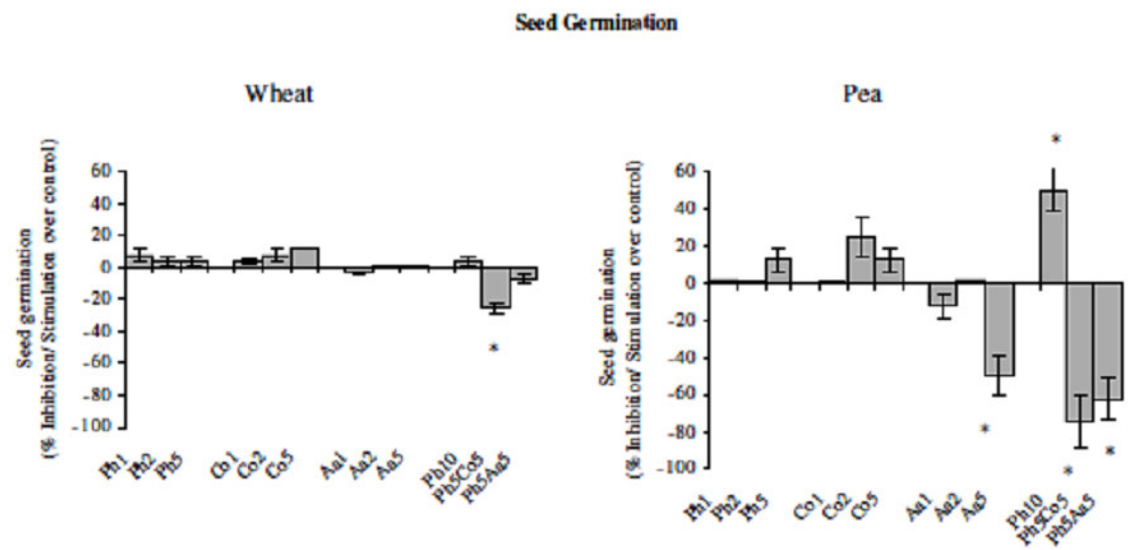

Figure 1. Influence of applied leaf-biomass of weeds (*Parthenium hysterophorus*, Ph; *Cassia obtusifolia*, Co; *Achyranthes aspera*, Aa) on the seed germination of wheat and pea. Digits (1, 2, 5, 10) suffixed to species codes (Ph, Co, Aa) indicate weight of leaf-biomass incorporated (g/Kg soil). Along Y-Axis the values < 0.0 indicate the inhibition (%) over control and values > 0.0 indicate the stimulation (%) over control. * indicates significant inhibition/ stimulation over control at the level of $p < 0.05$ according to Dunnett's test.

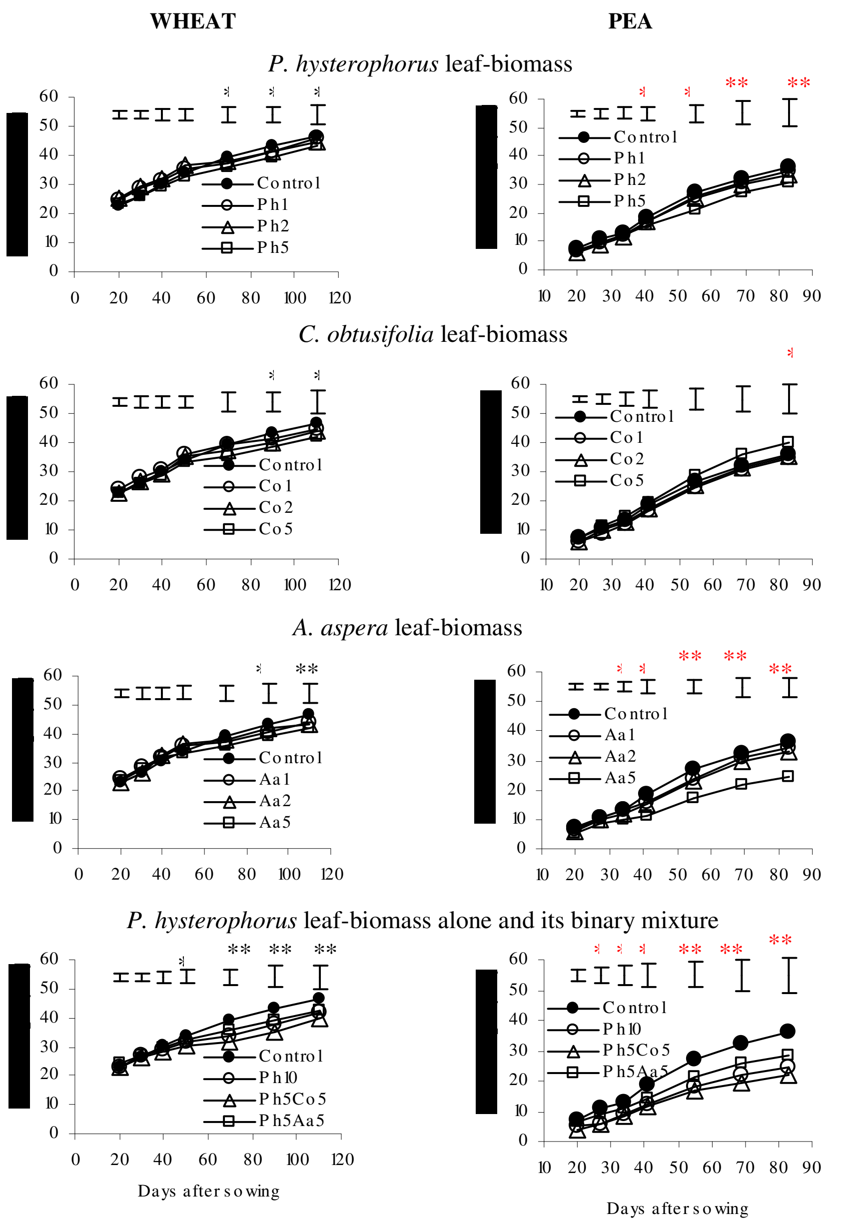

Figure 2. Effects of weed leaf-biomass (*Parthenium hysterophorus*, Ph; *Cassia obtusifolia*, Co; *Achyranthes aspera*, Aa) amended soils on shoot length of wheat and pea plants. Digits (1, 2, 5, 10) suffixed to species codes (Ph, Co, Aa) indicate weight of leaf-biomass incorporated (g/Kg soil). Bars indicate the LSD between treatment means (df = 32). * indicates significant at the level of $p < 0.05$ and ** $p < 0.01$.

under different soil amendments (Fig. 2). Plants in the amended soils generally showed higher initial growth compared to those growing in unamended soil. However, starting from 60-70 DAS, plants growing in control soil showed higher growth rates. In soils amended with relatively higher dose of leaf-biomass (5 g/Kg soil) reduced the wheat shoot length (7 - 10%). This reduction ranged from 3.2 cm (Ph5) to 4.5 cm (Co5). In soils amended with 10 g leaf-biomass of *P. hysterophorus* alone (Ph10) or its mixtures (Ph5 Aa5, Ph5 Co5), the shoot length of wheat plants showed growth reduction (4.2 cm - 6.7 cm). The Ph5 Co5 mixture showed a significant reduction in wheat shoot length (14 %) compared to the growth reduction recorded in Ph10 (11 %) and Ph5 Aa5 (9 %). Pea plants, on the other hand, showed contrasting growth trends in amended soils (Fig. 2). In soils amended with low amounts of Co leaf-biomass (<5 g/Kg soil), pea plants shoot length was not influenced over the control, while with 5 g/Kg soil (Co5), shoot length was increased (11 %). In Aa1, Aa2 and Aa5 the reduction in shoot length was 5, 9, 32 %, respectively. Ph and Aa leaf-biomass amended soils decreased the pea plant growth 1.8 cm (5 %) with increasing doses of biomass. However, the mixtures of leaf-biomass added to soil reduced the pea shoot length by 14.1 cm (39 %) in Ph5 Co5 and 7.6 cm (21 %) in Ph5 Aa5. The reduction in pea shoot length in Ph10 soils was 11.6 cm (32 %).

The number of leaves showed no response to weed-leaf biomass amendments. Responses of the basal diameter of the crop plants were significantly correlated with those of shoot biomass which showed significant responses to weed-leaf biomass. Figure 3 shows the shoot biomass of wheat and pea plants in various amended soils at maturity of pea (83 DAS) and wheat (110 DAS). The increasing soil leaf-biomass incorporation of Ph and Aa decreased the plant biomass. The increasing amount of leaf-biomass inhibited the growth of wheat plants in Co-amended soils (9 - 24% inhibition), but stimulated the pea plants growth (3 - 16%). The Ph5 reduced the shoot biomass of both crops (18 % in wheat and 32 % in pea) and Aa5 caused 27% inhibition in wheat and 34 % in pea. In soils amended with 10 g leaf-biomass of Ph or its mixture with Co or Aa, the growth of wheat and pea plants decreased significantly in Ph10 (32 - 38%) and to a higher extent in Ph5Co5 (37-44%) soils. The crop growth in Ph5Aa5 soils declined in comparison to control soils but was higher than that in Ph10. There was no additive effect of Ph and Co on shoot biomass in wheat (Table 1). However, the binary mixture of Ph and Co increased the shoot biomass in pea. The binary mixture of Ph and Aa resulted in antagonistic effects on shoot biomass of wheat and pea (Table 1).

In fallow soil (no crop), soil organic C increased from 6.08 mg/g (control) to 12.47 mg/g (Ph5 Co5) with various leaf-biomass doses (Table 2). This increase in wheat growth was from 5.73 mg/g (control) to 10.00 mg/g (Ph10) and under pea growth from 5.60 mg/g (control) to 9.82 mg/g (Ph10). The soils amended with 10 g weed-leaf biomass (either alone, Ph10 or in binary mixture, Ph5 Co5, Ph5 Aa5) showed much higher organic C content than control.

Soil organic C in all amended soils (except Aa1 soils) increased significantly with increasing amounts of leaf-biomass incorporated (Fig. 4). In soils amended with leaf-biomass of single species with dose of ≤5 g/Kg soil, this increase was highest in Ph-amended soils (11-64 %) and lowest in Aa-amended soils (2-52 %). Organic C levels of leaf-biomass amended soils, declined with crop cultivation compared to amended fallow soils. This decline was greater in pea compared to wheat.

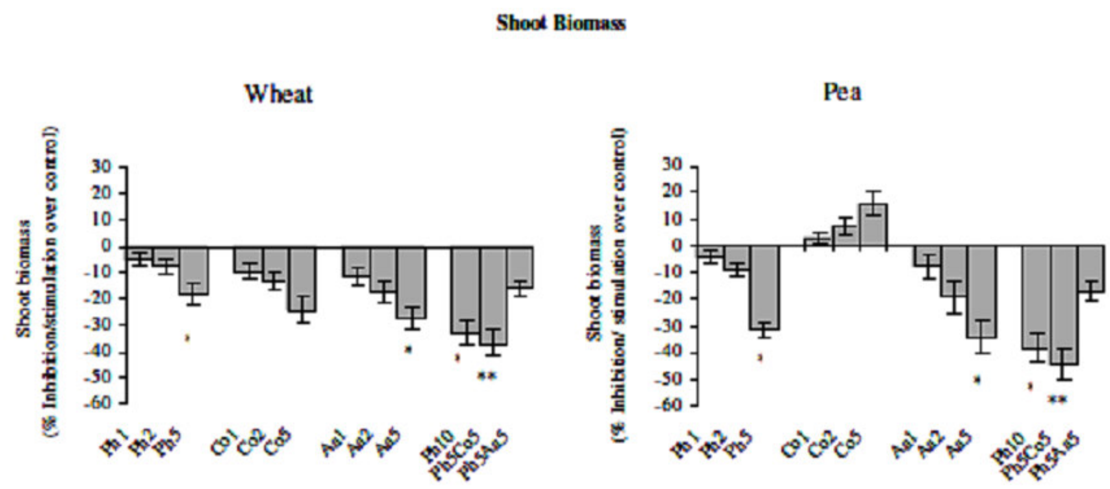

Figure 3. Effect of weed leaf-biomass (*Parthenium hysterophorus*, Ph; *Cassia obtusifolia*, Co; *Achyranthes aspera*, Aa) amended soils on shoot biomass of wheat (110 DAS) and pea (83 DAS) plants. Digits (1, 2, 5, 10) suffixed to species codes (Ph, Co, Aa) indicate weight of leaf-biomass incorporated (g/Kg soil). Along Y-Axis the values < 0.0 indicate the inhibition (%) over control and values > 0.0 indicate the stimulation (%) over control. Error bars represent standard error, * indicates significant inhibition/ stimulation over control at the level of $p < 0.05$ and ** $p < 0.01$, according to Dunnett's test.

Table 1. Effects of applied leaf-biomass of *Parthenium hysterophorus*, *Cassia obtusifolia* and *Achyranthes aspera* when applied alone and in binary mixtures of *Parthenium hysterophorus* with *Cassia obtusifolia* or *Achyranthes aspera* on shoot biomass of wheat and pea

Leaf biomass (g/Kg soil)	Shoot biomass (g/individual)	Measured Shoot biomass (% of control)	Expected value[a] (% of control)	Quality of interaction[b]
	Wheat			
Parthenium (5.0)	0.32	82		
Cassia (5.0)	0.29	76		
Achyranthes (5.0)	0.28	73		
Parthenium (5.0) + *Cassia* (5.0)	0.24	63	62	ns; Additive
Parthenium (5.0) + *Achyranthes* (5.0)	0.32	84	60	Antagonistic
	Pea			
Parthenium (5.0)	0.18	68		
Cassia (5.0)	0.30	116		
Achyranthes (5.0)	0.17	66		
Parthenium (5.0) +*Cassia* (5.0)	0.14	56	79	Synergistic
Parthenium (5.0) + *Achyranthes* (5.0)	0.22	83	45	Antagonistic

[a] as determined by the Colby's formula.
[b] Significantly different from measured value as determined by t-test ($p \leq 0.05$); ns = non-significant.

Table 2. Effects of applied leaf-biomass of weeds (*Parthenium hysterophorus*, *Cassia obtusifolia*, *Achyranthes aspera*) on organic carbon content (mg/g) of soil at 30 days after soil incorporation

Leaf biomass (g/Kg soil)	Wheat	Pea	Fallow (No Crop)
Control	5.73	5.60	6.08
Parthenium (1.0)	6.43	7.03	7.50
Parthenium (2.0)	7.17	7.33	7.63
Parthenium (5.0)	9.43	8.93	9.73
CD at 5%	0.33	0.38	0.26
Cassia (1.0)	6.53	6.00	6.93
Cassia (2.0)	7.08	7.42	7.52
Cassia (5.0)	9.02	8.60	9.25
CD at 5%	0.35	0.34	0.29
Achyranthes (1.0)	5.93	5.80	6.23
Achyranthes (2.0)	6.50	6.30	6.70
Achyranthes (5.0)	8.70	8.02	9.17
CD at 5%	0.32	0.35	0.25
Parthenium (10.0)	10.00	9.80	10.70
Parthenium (5.0)_+ *Cassia* (5.0)	9.37	9.82	12.47
Parthenium (5.0) + *Achyranthes* (5.0)	9.13	9.18	10.53
CD at 5%	0.39	0.41	0.32

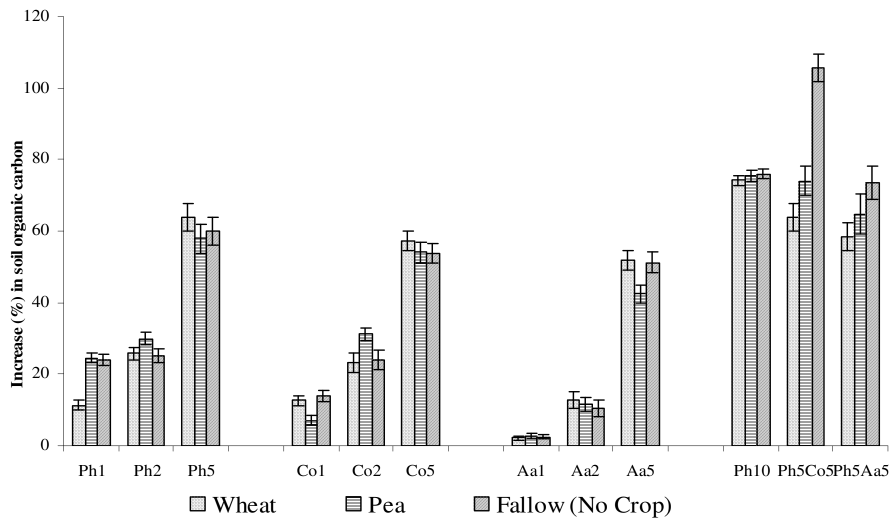

Figure 4. Increase (%) (mean ± S.E.) in organic carbon over control in soils amended with doses of weed-leaf biomass (*Parthenium hysterophorus*, Ph; *Cassia obtusifolia*, Co; *Achyranthes aspera*, Aa) under wheat, pea plants and fallow (no crop) condition. Digits (1, 2, 5, 10) suffixed to species codes (Ph, Co, Aa) indicate weight of leaf-biomass incorporated (g/Kg soil).

DISCUSSION

The present study revealed that the growth of crop plants was affected by weed-leaf biomass that enter the soil system after plant death. The leaf biomass contribution by the studied weeds (*P. hysterophorus*, *C. obtusifolia* and *A. aspera*) to litter input may be high in the areas of their preponderance as their leaf biomass comprised 42-47 % of their total shoot biomass (18,31). Crop seed germination, however, was not significantly affected by the applied amounts of leaf-biomass, particularly when added separately to soil in amounts less than 5 g/Kg soil). A possible explanation for this may be that the release of allelochemicals by decomposition of leaf-biomass was too slow to have affected germination during the short period for crop emergence. At higher amounts of leaf-biomass of *A. aspera*, an inhibitory effect on pea seed germination was detectable.

The observed crop growth inhibition or enhancement under the impact of various weed leaf-biomass amendments could theoretically be caused by the release of growth inhibiting/stimulating doses of allelochemicals during decomposition of the weed-leaf biomass (32, 33). Allelochemicals released are mostly water-soluble and released through leaching or through microbial decay (34). The adverse impact of *P. hysterophorus* on the growth of wheat and pea plants in this study was in accordance with its adverse effects reported earlier on *Cicer arietinum* L., *Raphanus sativa* L. (4) and *Brassica* sp. (36). This may be due to the release of water-soluble phenolic acids and the sesquiterpene parthenin (30) during decomposition. The non-nodulating leguminous weed *C. obtusifolia*, on the other hand, contains toxic anthraquinones: obtusifolin, obtusin, aurantio-obtusin and chrysoobtusin in its seeds (10, 14). An antimicrobial nature of the leaf extract of this weed has also been reported (14). However, its leaves are rich in proteins, calcium, phosphorus and magnesium (29), which may account for the observed growth stimulatory effect on the nodulating leguminous pea crop besides stimulation by low doses of allelochemicals.

In this study, the seed germination and crop growth distinctly declined in soils amended with higher amounts of weed leaf-biomass (10 g/Kg soil). The mixture of leaf-biomass of *P. hysterophorus* with that of *C. obtusifolia* (Ph5Co5) showed higher crop growth inhibition (dry weight reduction 37 % in wheat and 44 % in pea) compared to the inhibition recorded by *P. hysterophorus* alone (Ph10; 33 % reduction in wheat and 38 % in pea). Mixtures of allelochemicals may have a greater effect than the same concentration of the mixture compounds when used separately (6, 7). On the other hand, the growth inhibition was lower in Ph5Aa5 soils compared to Ph10. Thus, the present study showed a significant antagonistic activity of the binary mixture of leaf-biomass of Ph5Aa5. The interactive impact of binary mixture of leaf-biomass of Ph5Co5 varied with the crop. It acted synergistically to inhibit the growth of pea but acted additively for wheat. The synergistic impact of Ph5Co5 binary mixture to inhibit pea growth (although Co5 alone has stimulatory impact on it), is possibly suggestive of significantly altered soil environment under the impact of Ph-Co binary mixture through allelochemical interactions that may even adversely affect the growth of *P. hysterophorus* too. This, however, needs further investigation. *Cassia sericea* is reported to have a good 'antagonistic' effect against *P. hysterophorus* and can replace it from invaded areas (23). Therefore, the plant is often suggested as an eco-friendly strategy for the control of the invasive exotic weed (26). If the observed interaction between leaf-biomass of *P. hysterophorus* and *C. obtusifolia* also applies for *C. sericea,* an impairment of allelopathy of *P. hysterophorus* by leaf-biomass of *C. serica* may explain this 'antagonistic' effect against *P. hysterophorus* on simultaneously invaded sites.

In this study, a significant increase in soil organic C was recorded in soils amended with weed-leaf biomass compared to unamended control soils. This increase was highest in *P. hysterophorus* incorporated soils possibly due to a faster decomposition. Thus, even one of the world's worst invasive species contributes significantly to the organic C pool of dry tropical disturbed soils. This has also been observed at natural *P. hysterophorus* infested soils along the Kali river by Gupta & Narayan (19) who reported an even higher soil organic C content (0.8-1.1 %) and C:N ratio (28-31). The increased organic C in amended soils may be attributed to a lower microbial activity in the rhizosphere presumably caused by antimicrobial phytochemicals released by the weeds (42).

Although the weed-leaf biomass add to the organic C pool of the soil, they may still adversely impact the growth of associated plants by a biochemical impact of allelochemicals such as parthenin (30), achyranthine (20) or alkaloids/secondary metabolites/toxins released into the soil by the weeds. Thus, the currently observed increase/decrease in crop growth by weed-leaf biomass amendments of soils should rather result from allelochemicals released into the soil than from soil organic C status. However, under natural conditions, it is often difficult to separate the effect caused by allelochemicals or by an altered availability of nutrients. Thus, both aspects could be alone or simultaneously responsible for the results observed in this study. The immense value of organic C addition to the soil by decomposing weed-leaf biomass has promising weed management implications, particularly where the impact of allelochemicals is short-lived.

CONCLUSIONS

This study revealed that leaf-biomass of weeds (*P. hysterophorus, C. obtusifolia*, *A. aspera*) significantly improved the soil organic C pool and influenced the crop growth. However, their impact was crop specific and depended on doses and quality of leaf-biomass (weed type). The mixtures of *P. hysterophorus* had variable effects (additive, antagonistic and synergistic) on the crop growth, thus, weed biomass may have potential in weed management.

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