

Comparison of Estimation of Soil Weed Seed Bank of Agroecosystem of Moist Tropics India

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Abstract – *The soil weed seed bank is defined as the mature viable weed seed stocks existing in the soil surface or buried in the soil or litter in an agroecosystem. The information is lacking for many weed species and cropping systems of India and in other agroecosystem of the world determining weed population dynamics. The aim is to evaluate weed seed density in soil seed bank in terms of comparison of two method of seedling germination to get information which method is more appropriate for seed bank estimation for proper weed management in moist tropical agroecosystem. The higher seed density were found in seed extraction method than seed emergence method. The result revealed that both methods are necessary for estimation of soil weed seed bank. In future studies, emergence methods are recommended as basis to evaluate the short-term outcome of the revegetation process if the soil-stored seed bank is used. Both methods were complementary to detect the species composition. Therefore it is suggested that both methods are necessary to describe the seed banks and improve the interpretation of the results.*

Keywords: *Soil weed seed bank, seed extraction, wet sieve method, seed emergence method, Agroecosystem, Moist tropics.*

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INTRODUCTION

Agroecosystem is a key motor of global economy and support livelihoods and subsistence of largest number of the people worldwide. Agriculture is the cultivation of land for the advantage of selected species including agricultural crops, livestock, tree crops and grazing lands. The productivity of agricultural ecosystems depends on numerous species, such as soil micro-organisms, pollinators, predators of agricultural pests and the genetic diversity of the goal oriented crops and livestock. The goal oriented crops in agricultural lands play a critical role in food security for many low-income farmers and rural people, as animal feed, fuel, raw materials for processing, and to provide supplemental food. Agro-biodiversity is the diversity related to the agroecosystem and encompasses the variety and variability of plants i.e. crops and weeds both. So it is important to study soil weed seed bank in agroecosystem of moist tropics. So as from above discussion we have noticed how the agroecosystem of world is so important for human, animals and the environment, so proper weed management is necessary in agroecosystem to maintain agro-biodiversity and its sustainability for future generations for food production, and other economical purposes (herbal medicine, fodder, and fibers etc.)

The soil weed seed bank is defined as the mature viable weed seed stocks existing in the soil surface or buried in the soil or litter in an agroecosystem. Weed plants after maturation shed their seeds and these weed seeds ultimately accumulated in the soil profile which form weed seed bank in the soil (Hussai et al., 2017). The weed seed bank is recognized as the primary source of annual weeds in agroecosystem. The population dynamics of weeds in agroecosystem are largely influenced by the growth of crop plants as well as their own growth which are determined by the physical environment (Misra et al., 1995). The study on weed population ecology in crop fields encompasses the analysis of the dynamics of the soil weed seed bank and the plant populations in time and space (Misra et al., 1995). Many factors such as tillage, ploughing, use of herbicides and fertilizers, soil properties and weed control methods have various impacts on weed population. Weeds rapidly spread into new areas due to its seed germinating power and have large seed production from single plant. Soil seed bank is presence of viable seed buried into the soil or unto the soil and in agroecosystem its study is known as weed seed bank. An understanding of soil seed banks dynamics of weed ecology is important for effective vegetation and weed restoration of agroecosystem of moist tropics. The information is lacking for many

weed species and cropping systems of India and in other agroecosystem of the world determining weed population dynamics.

The seed extraction method of removing seeds from the soil and counting them might be the most accurate (Mesgaran et al., 2007) time, money and personnel limitations result in the continuing use emergence or germination method for assessing seed banks (Kalamees and Zobel 2002; Ris Lambers et al. 2005; Clarke and Dorji 2008; Espeland et al., 2010). The extraction method from soil is both time and labour consuming and results are influenced by sampling techniques, the time sampling and methods to determine seed numbers (Gross 1990; De Villiers et al., 1994). The emergence method underestimate viable seeds and extraction method overestimate the counted seeds. The aim is to evaluate weed seed density in soil seed bank in terms of comparison of two method of seedling germination to get information which method is more appropriate for seed bank estimation for proper weed management in moist tropical agroecosystem. Such comparisons are necessary to determine accurate methods for estimating weed seed characteristics of soil seed bank (Gross 1990).

MATERIAL AND METHODS

Study area and climatic conditions

The study area is located (between 26° 13' and 27° 29'N latitude and 83° 05' and 83°56'E longitude) in Gorakhpur district in a tropical moist region of eastern Uttar Pradesh, India. All three seasons summer (March-June), rainy (July-October) and winter (November-February) is prominent in the area respectively. Total annual rainfall ranges between 1850-2000 mm (85% rainfall during monsoon from July to October). Temperature, humidity and precipitation always fluctuate with climatic change. Mean maximum temperature is 27-32°C and mean minimum temperature is 15-20°C. Mean maximum and minimum humidity is 85% and 30%, respectively. The soil is Gangetic alluvium and color is blackish grey, texture is sandy loam and clay. Soil pH is slightly neutral and soil is fertile. The farmers are harvesting crops since last 80 years. The main crops are rice in rainy season, wheat and mustard in winter season, maize during transition period along with vegetable crops. The agricultural land practice has been selected near Ramgarh village which is situated near the Kusmahi forest range, the villagers sowed different varieties of seasonal crops and vegetables but for this study crops such as rice, wheat and maize, mustard and vegetable were selected.

Soil collection

In all ecosystems three permanent plots (100 m x 100 m) with two sub plots (50 cm x 50 cm) in each plot, were marked. Within each subplots soil was

excavated from a 20 cm x 20 cm area in three successive 10 cm depths (0-10 cm, 10-20 cm and 20-30 cm). Before collection of the soil sample litter accumulated on the surface was removed. These samples were collected twice during two annual cycles once after the rainy season (October-November) and other after winter season forthcoming summer-rainy (May- June). Depth samples of soil (6 sub-plots x 3 depths per plot; each corresponding to 20 cm x 20 cm, 10 cm depth) were air dried and weighted.

Soil seed bank estimation

All soil depth samples were used for estimating the seeds of soil seed bank by two methods; first based on direct count of seeds, seed extraction or wet sieve method (Thompson et al., 1997) and second based on emergence of seedlings (Ter Heerdt et al., 1996) in soil collected from agroecosystem in the same soil samples of same depths.

Seed extraction or wet sieve method

In the first method, the seeds were extracted by a sieving/ flotation method using 200 gm soil samples (Konstantinovic et al., 2011). Each depth sample of soil was analyzed in triplicate, totaling to 72 samples (6 sub-plots x 3 depths x 3 replicate) in a season. The soil sample was crushed gently and suspended in water to facilitate the dissociation of the soil aggregates. The suspension was poured through a set of four tiers of sieves in a large tub. The upper most sieves had 1 mm pore size, the second 0.5 mm, third 0.2 mm and lower 53 µ. Seeds retained on different sieves were transferred to a filter paper, air-dried and sorted by species and identified and then counted. The identification of seeds was confirmed by comparison with seeds of different species collected during the preceding annual cycle from different study sites and manually from book named Identification of Seeds (Martin & Barkley 2005). The seeds, which are crushed or damaged by parasites attack or by any other factors, were excluded. The number of seeds estimated in 200 gm soil sample was adjusted to depth sample weight and expressed on unit area basis.

Seedling emergence method

In second method, a weighed portion of each depth sample of soil was transferred to a flat, shallow earthen pots filed to about 1 cm below its brim. The earthen pots were placed in open field in the Botanical Garden of Department of Botany, Banaras Hindu University. Each earthen pot was periodically sprayed with water to provide adequate soil moisture for seed germination, as per seasonal requirement. Emerged seedling were identified, counted and removed from the pots as 15-day intervals. Few seedlings of each species were

transplanted to fresh pots and allowed to grow till flowering to confirm their identification. When seedling emergence ceased, the soil in earthen pots was lightly worked, occasionally sprayed with water and emerged seedling count still continued, in all counting were made at 15 day intervals during both seasons, while seedling emergence continued till completion of one annual cycle (Luscheri 2003). Few species were emerged in all samples during throughout the season and each was counted from time to time. Considering the fact that earthen pots were placed in open field condition, a set of control earthen pots was also established during each season to note the seedling emergence due to local seed rain. For this purpose, representative soil samples of each depth (0-10 cm, 10-20 cm and 20-30 cm) were autoclaved at 200°C for 4 hours to ensure the absence of any viable seeds. The autoclaved soil samples of each depth were placed in earthen pots (6 replicates). Number of seedling emerged in control pots were subtracted from the respective seedling numbers in different depth samples. Finally, the estimate of number of seedlings (seed density m^{-2}) recorded in earthen pot soil was adjusted to total weighed of soil in the depth sample with bulk density of that soil (Elsafori et al., 2011). The depth sample data, corresponding to 20 cm x 20 cm area, was further converted to per m^2 land area basis.

RESULTS AND DISCUSSION

Soil seed bank (seed extraction or wet sieve method)

The size of soil seed bank up to 0-30 cm depth determined by wet sieving methods ranged from 52020-56520 in agroecosystem. In this method eight weeds were have higher contribution in seed density identification others are not least contributed or not identified. A total of eight species were identified in agro-ecosystem by wet sieving method. Total seed density in agroecosystem was maximum for *Euphorbia hirta* (3800-3845 seeds m^{-2}) and minimum for *Solanum nigrum* (187-674 seeds m^{-2}) in both annual cycles (Fig. 1). Seed density of other species *Ageratum conyzoides*, *Amaranthus viridis*, *Chenopodium album*, *Cynodon dactylon*, *Cyperus* spp., and *Dichanthium annulatum*, ranged in soil seed bank as 604-640 seeds m^{-2} , 627-657 seeds m^{-2} , 682-751 seeds m^{-2} , 3546-3724 seeds m^{-2} and 2457-2846 seeds m^{-2} , respectively in both annual cycles. *Solanum nigrum* showed greater fluctuation in seed density, as it had 674 seeds m^{-2} in first annual cycle and 187 seeds m^{-2} in second annual cycle. Unidentified species ranged between 2946-3746 seeds m^{-2} in both annual cycles.

Soil seed bank (seedling emergence method)

During the annual cycles, total number of seedlings emerged from 0-30 cm depth ranged from 22750-25050 seedlings m^{-2} in agroecosystem. Total 22

weeds were recorded during study period in seedling emergence method. The total seedlings emergence of *Euphorbia hirta* was maximum (4535 seedling m^{-2}) in all seasons of both annual cycles (Fig. 2). Beside this, in agroecosystem, *Blumea lacera*, *Cynodon dactylon*, *Cyperus rotundus*, *Dichanthium annulatum*, and *Parthenium hysterophorus* contributed maximum (17700 seedlings m^{-2}) because they germinate throughout entire season. Winter annuals (*Ageratum conyzoides*, *Anagallis arvensis*, *Chenopodium album*, *Echinochloa colona*, *Eragrotis unioides*, *Melilotus alba*, *Phalaris minor* and *Solanum nigrum*) were germinated in their respective (winter season) germination time and contributed 5023 seedlings m^{-2} in seed bank. Majority of the species (*Ammania baccifera*, *Blumea lacera*, *Boerhaavia diffusa*, *Brachiaria ramosa*, *Commelina benghalensis*, *Cynodon dactylon*, *Cyperus kyllingia*, *Cyperus rotundus*, *Dichanthium annulatum*, *Digitaria ciliaris*, *Euphorbia hirta*, *Ludwigia parviflora* and *Parthenium hysterophorus*) germinated during rainy season and contributed 13339 seedlings m^{-2} but a few species (*Blumea lacera*, *Boerhaavia diffusa*, *Cynodon dactylon*, *Cyperus rotundus*, *Dichanthium annulatum*, *Euphorbia hirta* and *Parthenium hysterophorus*) were germinated in summer season contributing 6118 seedlings m^{-2} in soil seed bank. *Ludwigia parviflora* (181 seedlings m^{-2}), *Melilotus alba* (318 seedlings m^{-2}) and *Solanum nigrum* (388 seedlings m^{-2}) were present in rainy and winter seasons of first annual cycle. *Boerhaavia diffusa* (775 seedlings m^{-2}) was present in all seasons of first annual cycle and not recorded in second annual cycle.

Comparison of seed extraction or wet sieve and seedling emergence method

In agroecosystem, the ratio (wet sieve/seedling emergence) first decreased in 10-20 cm soil depth then increased in 20-30 cm soil depth (Table 1). Ratio of seed count and seedling emergence was recorded from 0.97 to 2.38. Maximum ratio (2.38) was found for identified and unidentified species in 0-10 cm soil depth and minimum for *Cyperus* spp. (0.97) in 0-10 cm soil depth. The differences in seed number obtained by both methods described the role of seed dormancy in the emergence method. Roberts (1981) found that seedling emergence gave an estimate equivalent to 38% (75% when the species with highest seed numbers were excluded) of the viable seeds recovered by separation. The species wise seed bank in different depths of soil estimated through direct counting of seeds (seed density) by wet sieve method and counting of emerged seedlings by seedling emergence method and their ratios in different ecosystems were presented (Table 1). The greater ratios were recorded for *Amaranthus viridis* however ratio fluctuation were seen from 0-30 cm soil depth. Seed bank estimation by wet sieving and seedling emergence method showed significant wider variation. Wet sieving method of soil seed bank estimation was significantly higher

than seedling emergence method of seed bank. The ratio of two seed bank estimation in soil depth may increase or decrease in most of species. Soil seed bank estimate by both methods was 44% in agroecosystem. The soil seed bank by these two methods got high ratio in present study whereas low emergence ratios was reported by several workers (Jensen 1969; Ball & Miller 1989) as this study suggest that it is possible to achieve high ratio under appropriate growing condition in some soil. Most of the dominant species (*Cynodon dactylon*, *Cyperus* spp., and *Euphorbia hirta*) showed considerable higher differences in ratio between two methods. The high ratio suggested comparative estimates of soil seed bank in moist tropics because seed germination of most species occurred entire season in a year whereas high ratio indicated that both estimates of seed bank differ significantly.

DISCUSSION

Agricultural biodiversity (the biodiversity associated with agricultural ecosystems) is indispensable for plant stability, and therefore sustaining crop production, food security and livelihoods for everyone. Agroecosystem is the backbone of India in terms of economic growth which influences GDP of the country and is a most important type of goal oriented ecosystem which is evident from land creations wellbeing, and maintenance of weed species diversity coupled with crops diversity which provides food to human beings.

Weed seed bank research in present day is significant topic to develop successful viable and sustainable weed management systems for many areas of crop field. The knowledge of weed seed banks is necessary to carry out weed population dynamics and establish proper weed management options to prevent both crop for food and future security and weeds for medicinal and economic purposes and. There are less disturbances and grazing in their agricultural land because farmers protect and managed their crops and other economical weeds which are used as fodder, fuel and as medicine. The evaluation of weed seed bank in an agroecosystem is important for weed management. The success of weeds in an agroecosystem is mainly due to their high diversity and enormous capacity to produce seeds for the propagation of off springs (Dekker 1997). The study on weed population ecology in crop fields encompasses the analysis of the dynamics of soil seed bank and plant populations in time and space (Misra et al. 1995), spatial and temporal variations in the soil seed bank and seasonal and depth wise variations in present study. The seed bank is important component of agroecosystem is the primary source of future weed populations serving as unique resource for predictive weed management purposes (Forcella et al. 1997). Many factors such as tillage, ploughing, use of herbicides and fertilizers, soil properties and weed control methods have

various impacts on weed population. Weeds rapidly spread into new areas due to its seed germinating power, seed rain/seed dispersal, movement by animals and have large seed production from single plant. Weed management is as old as agriculture itself and weeds are those plants which are harmful, interfere with agricultural operations, increase labors, add input to the cultivation and reduce the crop yield (Sen 2000). Weeds are contributing factor in crop production due to yield loss but sometimes these weeds are beneficial for human in form of herbal medicine, fibers, ropes, forage and fodder as found in agroecosystem of this study. Weed communities co-evolve with cropping systems, allowing the populations to adapt to highly, regularly disturbed environments (Martinez-Ghersa et al. 2000). Weed seed banks can be memory of a weed community especially those communities dominated by annual weeds (Cavers 1995) as in our result we found dominance of annual weeds because they generally persist in the soil for many years (Thompson et al. 1997). Analysis of weed seed bank composition based on relative abundance may have a higher predictive value on weed community dynamics than one based on density because emphasis was given to species importance in the community rather than to their absolute densities (Barberi & Cascio 2001).

Sieving method detected greater seed density m^{-2} and species richness and diversity of weed seed bank compared with seedling emergence method (Hussain et al., 2017) same result also recorded in present study. The differences in species diversity of soil seed bank between the two methods were in agreement to the results of Price et al. (2010) who found large differences in species composition of soil seed bank between both sieving and seedling emergence method at various soil depths same as in present study of agroecosystem. The main advantages of the seedling emergence technique are that the effort required is spread over a period, each seedling represents a viable seed, and seedlings are usually easier to identify than seeds (De Villiers et al., 1994). This method is therefore usually preferred for monitoring long-term experiments and for studying seasonal changes in the seed bank. On the other hand, separation by physical seed extraction means would be expected to reveal many more seeds than seedling emergence, and seed numbers may be overestimated unless they are adjusted for viability (Gross 1990). However, for many species it is not possible to distinguish between viable and non-viable seeds in the seed extraction samples (Roberts 1981). Seed extraction methods have the advantage that the results are not influenced by differences in germination requirements and dormancy, but the accuracy of these methods is variable and species with small or cryptically coloured seeds may be missed. Seed extraction methods are, however, extremely laborious and impracticable for large-scale studies, especially

those involving small-seeded species as in this case. Seed size is another important factor determining the most suitable method to estimate the size of the seed bank of a specific area. Poiani and Johnson (1988) found that the presence of many large seeds made the seed extraction method unreliable, whereas Gross (1990) found small seeds did the same. Roberts (1981) found the problem of isolating seeds to be more difficult when, as is usual, a range of species was present which differed in seed size. In this study, the majority of seeds were found to be very small, which complicated seed isolation, identification and determination of viability after flotation had been completed (De Villiers et al., 1994). With the emergence method a larger proportion of smaller seed species could be detected, whereas the extraction method had the enhanced ability of detecting tree and shrub species which had larger seeds. Pierce and Cowling (1991) reported that species differences in estimates were a function of seed bank size. Species with larger seed banks gave higher estimates by counting than species with fewer soil-stored seeds, which could easily be overlooked during seed counts. The germination of seeds through emergence method varies due to length of time of germination (ranges from weeks to years), pretreatment and preparation, temperature, light, watering regime, watering direction rates, availability, microfauna, fungi and soil texture (Espeland et al., 2010). The seed extraction method may prefer for large seeded plants while seedling emergence method is for small seeded plants and long term monitoring. In future studies, emergence methods are recommended as basis to evaluate the short-term outcome of the revegetation process if the soil-stored seed bank is used (Gonzalez and Ghermandi 2012). Both methods were complementary to detect the species composition. Therefore it is suggested that both methods are necessary to describe the seed banks and improve the interpretation of the results.

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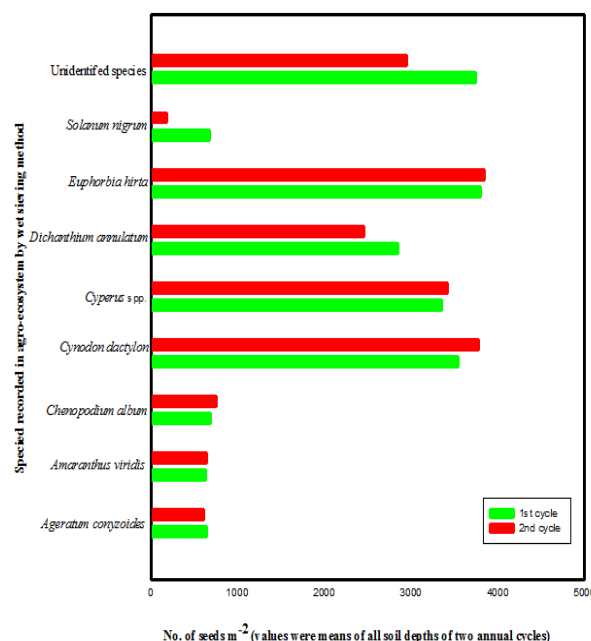


Fig. 1 Total number of seed density (seeds m⁻²) of each species in soil weed seed bank recorded in agroecosystem of moist tropics, UP, India by seed extraction or wet sieving method of two annual cycles (2007-2009).

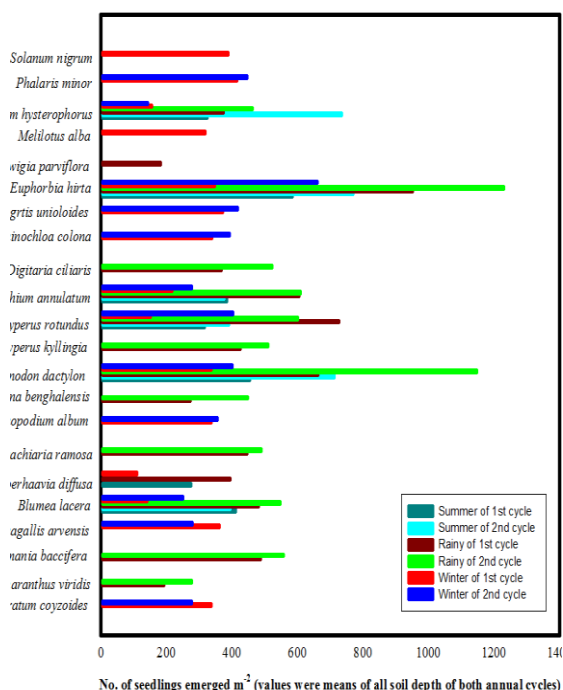


Fig. 2 No. of seedlings emerged (seedlings m^{-2}) of each species by seedling emergence method in two annual cycles (2007-2009) in agroecosystem of moist tropics, UP, India. (Values are means two annual cycles)

Table 1 Comparison between two estimates of species composition of soil seed bank in the agroecosystem of moist tropics, UP, India; values are mean of two annual cycles (2007-2009); WS = Seed bank by seed extraction or wet sieving method, SE = seed bank by seedling emergence method, W/S = ratio between estimates of two methods (Soil depths in cm).

Species	Depths (cm)								
	0-10			10-20			20-30		
	WS	SE	W/S	WS	SE	W/S	WS	SE	W/S
<i>Ageratum conyzoides</i>	906.7	428.8	2.13	565.8	292.5	1.93	392.5	199.7	1.97
<i>Amaranthus viridis</i>	983.3	316.0	3.11	558.2	237.0	2.35	355.2	150.2	2.37
<i>Chenopodium album</i>	1071.0	464.5	2.30	675.3	341.0	1.88	403.8	231.7	1.74
<i>Cynodon dactylon</i>	6622.7	4097.0	1.62	2754.2	1101.1	2.50	1604.7	872.1	1.84
<i>Cyperus</i> spp.	5079.0	5262.0	0.97	3104.8	1125.5	2.00	1972.7	1116.5	1.77
<i>Dichanthium annulatum</i>	3958.5	4305.0	1.08	2413.0	1404.3	1.72	1585.2	994.0	1.59
<i>Euphorbia hirta</i>	6972.3	4243.0	1.64	2654.5	1589.2	1.67	1941.7	1117.5	1.73
<i>Solanum nigrum</i>	657.6	285.8	2.30	3996.7	163.3	2.45	235.0	132.8	1.76
Others identified and unidentified	5104.	2137.0	2.38	3121.5	1645.4	1.89	214.2	1055.3	2.03

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