INHIBITORY AND STIMULATORY EFFECTS OF ROOT EXUDATES IN TWO VARIETIES OF RICE

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ABSTRACT

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The (allelopathic) interaction between two varieties of rice (Oryza sativa) seedlings Subarna and Pankaj was studied. Root exudates (RE) of Subarna inhibit the germination and growth of the seedlings of Pankaj and vice-versa. RE of both Subarna and Pankaj contain phenolic compounds, amino acids and presumably a fatty acid. The putative fatty acid appears 15-20 days following germination and disappears after two months. Only one phenolic was detectable in the later stages and two others in young seedlings. The Rf values of these three putative phenolics show that none of these is among the eight standards, namely: syringic acid, caffeic acid, ferulic acid, 3-4 dihydroxy benzoic acid, vanillic acid, 4-hydroxy benzoic acid, coumaric acid, and sinapic acid. The synergistic effect is inhibitory but when chromatographically separated two inhibitors and three stimulators have been found in RE of two-month old Subarna.

KEY WORDS: Allelopathy. Phenolics. Rice (Oryza sativa). Root exudates.

INTRODUCTION

Plants liberate different metabolites through their root system into the soil and such substances may significantly affect the growth of other neighboring plants (Bonner, 1950; Bonner, 1960; Woods, 1960; Grab, 1961). Molisch (1937) first coined the term allelopathy to describe the action (inhibitory and stimulatory) of a species of higher plant on another effected through root exudates (RE). Grummer (1961) used the term kolines for chemicals produced by higher plants and effective against other higher plants. Specific examples of such chemicals have been discovered (Bonner, 1950;

Bonner 1960; Woods, 1960). Numerous examples of allelopathy have been reviewed by Rice (1984). However, little investigation has as yet been undertaken on the stimulators of rice (Oryza sativa) RE. Stevenson (1967) and Chandramohan, et al (1973) reported a number of inhibitors in rice RE. Some of these are alipathic acids (Stevenson, 1967) and phenolics like p-coumaric acid, p-hydroxy benzoic acid, vanillic acid (Chandramohan, et al, 1973). Sadhu and Das (1969, 1971) isolated, with the help of paper chromatography, the inhibitory and stimulatory substances from the RE of two varieties of rice (Rupsail and CB-1). Tapaswi, et al (1991) analyzed the results of field experiments with Pankaj and Subarna and suggested the presence of both inhibitory and stimulatory compounds in the RE. In this paper, the chemical nature and also the inhibitory and stimulatory effects of RE of Pankaj and Subarna in the laboratory are reported.

MATERIALS AND METHODS

Three varieties of rice were used, namely: Subarna, an indigenous long duration tall aman variety (developed in ISI farm at Giridih, Bihar); Pankaj, a long duration high yielding dwarf variety; and IET 1444, used for bioassay on the sections of the chromatogram only.

RE was collected for 2-4 days according to the following procedures:

a) *Small seedlings* - A filter paper of width spanning that of a 500 mL beaker was dipped in 100 mL water in such a manner that part of it lay above the water level. Then 50 sterilized seeds were pressed against the moist paper (above the water level). Because of the serrated edges of rice grains, they remain stuck under these conditions.

b) *Larger seedlings (infloresecence stage)* - Ten seedlings were put in a 500-mL conical flask containing 300 mL water.

Effects of RE of one variety on germination and growth of another variety were detected by bioassay. Seeds were sterilized with 0.1% mercuric chloride solution, washed with distilled water and were placed on a filter paper in a petri dish. A proper control was maintained by treating equal volume of distilled water (instead of RE). After 3 days, nearly 100%

germination was noticed. Shoot and root length in the control and treated sets were then measured.

Isolation and detection of the putative active compounds in the RE were attempted with the help of paper chromatography. Whatman paper no. 3 was used to develop the chromatograms with three different solvent systems. For phenolic compounds, the solvent was n-butanol:formic acid:water (5:2:5, upper phase collected 24 h after settling); for fatty acids, the solvent was methanol:ammonia (99:1); and for amino acids, n-butanol:acetic acid:water (4:1:1).

Phenolic compounds were stained using silver nitrate solution, prepared by adding 1 mL of saturated aqueous silver nitrate, with stirring, to 20 mL acetone. The solution was then treated dropwise with water just until the precipitated silver nitrate has dissolved (Stahl, 1969).

For staining fatty acids, a solution of 0.03% methyl red in 0.05 (N) borate buffer (pH 8) was used; for staining amino acids, 0.2% ninhydrin solution in butanol.

Bioassay was done on the preparative chromatogram; 1 mL of RE of Subarna was used for preparative chromatography (using solvent system of n-butanol:formic acid:water = 5:2:5, upper phase). Thé chromatogram was cut into equal slices. The width of the slices was determined from the stained spot (left vertical section in region 1). Height of the chromatogram was 19 cm. Twenty five seeds of Pankaj were placed on each piece including control.

Seeds were arranged on each section of the paper divided verticallly near the middle so that one was slightly longer. In the shorter section, 12 seeds were placed in two rows (6 each) and in the longer, another single seed was put beside the two rows. An approximately equal-sized piece cut out from the blank run under identical conditions served as control. Shoot and root lengths were measured after 3 days.

RESULTS AND DISCUSSION

Bioassay

RE of Subarna on Pankaj

Two sets of measurements were performed: one for the treated set with RE of Subarna on the germination of Pankaj seeds, and the other for control. After 3 days, 100% germination was noticed. Shoot and root length in the control and treated set were then measured using a millimeter scale.

Table 1 shows inhibition in a treated set. Four such sets (50 seeds each) were examined.

Table 1. Percentage inhibition of shoot and root length in germinating Pankaj seeds treated with Subarna RE.

Observation	Control		Treatment		Percentage Inhibition	
	SL[1]	RL[2]	SL	RL	in SL	in RL
1	54.9	78.2	46.7	56.0	15	28
2	50.5	85.9	37.6	70.7	25	18
3	49.8	30.6	40.0	37.8	19	25
4	47.0	69.4	38.4	55.2	18	20

[1]SL = shoot length, mm; [2]RL = root length, mm.

Table 2. Percentage inhibition of shoot and root length in germinating Subarna seeds treated with Pankaj RE.

Observation	Control		Treatment		Percentage Inhibition	
	SL[1]	RL[2]	SL	RL	in SL	in RL
1	46.1	59.8	41.8	46.0	9	23
2	87.5	53.3	72.9	45.2	16	15
3	38.0	60.2	31.4	44.8	17	25
4	60.2	66.9	49.8	57.4	22	14

[1]SL = shoot length, mm; [2]RL = root length, mm.

RE of Pankaj on Subarna

Two sets of measurements, as above, were performed and 100% germination was noticed after 3 days. Shoot and root length in the control and treated set were then measured as before. Table 2 shows the inhibition in a treated set (50 seeds/set).

Results indicate that RE of both Subarna and Pankaj inhibit the shoot and root lengths of the seedlings of the other variety.

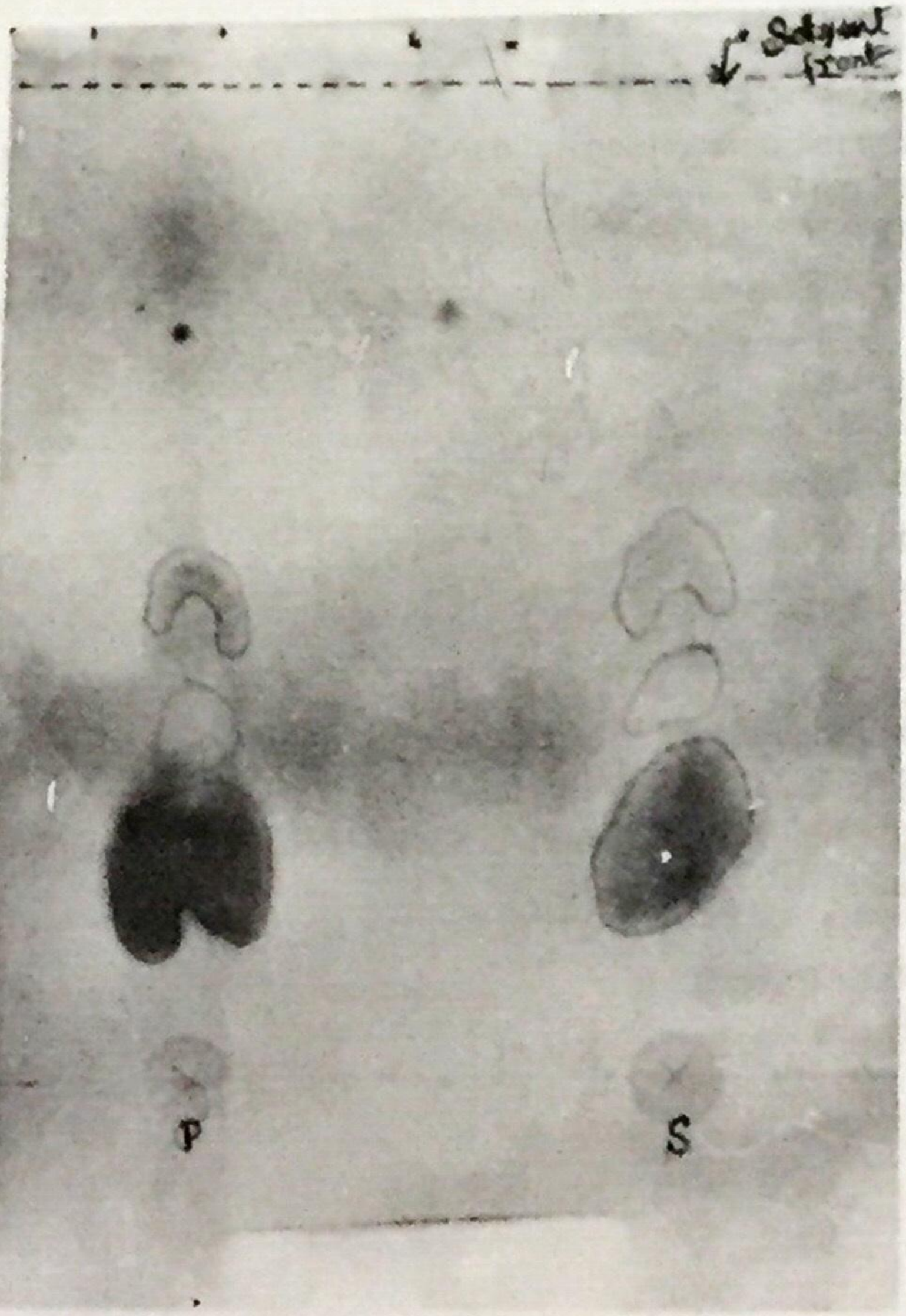

Figure 1. Chromatographic separation of root exudates of Subarna (S) and Pankaj (P).

Paper chromatography

Presence of phenolic compounds

In both Pankaj and Subarna RE 3 spots with low Rf were visible after staining with $AgNO_3$ reagent (Figure 1). This was detected in 15-20 day-old seedlings and only one spot was found in 2-mo old seedlings and up to near-inflorescence stage. Eight standard phenolics (syringic acid, caffeic acid, ferulic acid, 3-4 dihydroxy benzoic acid, vanillic acid, 4-hydroxy benzoic acid, coumaric acid and sinapic acid) were seen to have far higher Rf values in the same solvent. Presence of phenolic substances in the RE was also confirmed by adding freshly mixed solution of 1% potassium ferricyanide and 1% ferric chloride to it (Harborne, 1968).

Presence of methyl red positive substance

A single methyl red positive spot was found in case of 15-20 day old seedlings of both Pankaj and Subarna RE, but was absent in all seedlings below this age and also after the age of 2 mos up to inflorescence stage (Figure 2).

Presence of amino acid

Four ninhydrin positive spots were noticed in the RE of Subarna and three spots in the RE of Pankaj in both young and 2-mo old plants (Figure 3).

Chromatographic isolation and bioassay

One mL of RE of Subarna was used for chromatography (Solvent is n-butanol:formic acid:water [5:2:5], upper phase). One vertical piece of the chromatogram was stained with $AgNO_3$ reagent. After having gained a preliminary idea of the location of stimulators and inhibitors with the help of orientation experiments, the remaining larger unstained portion of the chromatogram was divided into 9 parts (Figure 4). Twenty five seeds of Pankaj were placed on each piece, including control. An approximately equal-sized piece cut from a blank run under identical condition served as control. Shoot and root lengths were measured after 3 days.

Two inhibitors in regions 2, 3 and 7 were noticeable. Inhibition was, on the average, 37%. Stimulators were observed in 1, 4, 5, 6, 8 and 9. Stimulation varied from 19% to 97%.

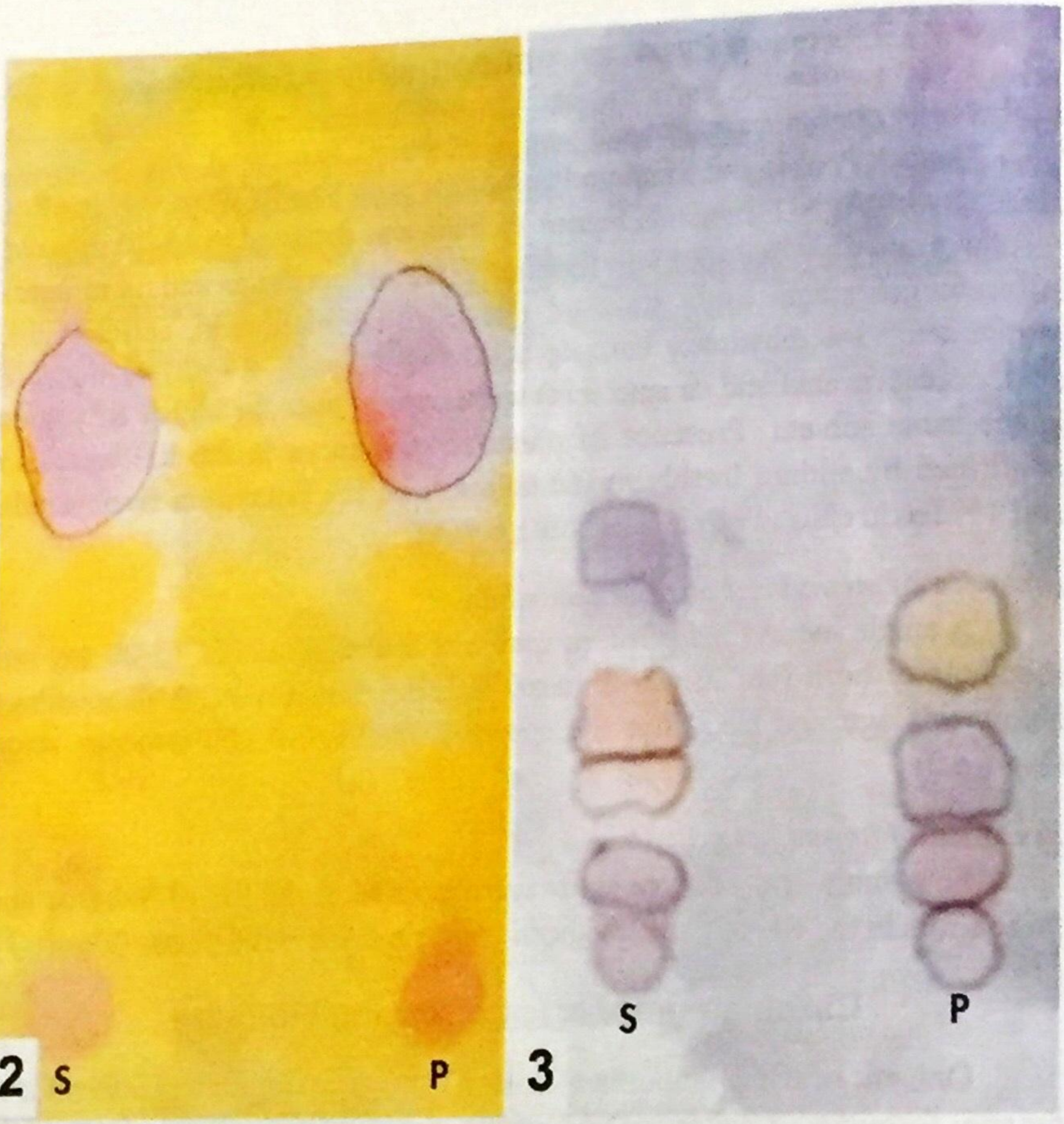

Figure 2. Chromatographic separation of root exudates of Subarna (S) and Pankaj (P) using as solvent methanol:ammonia solution (99:1). Stain was 0.03% methyl red in 0.05 (N) borate buffer.

Figure 3. Chromatographic separation of root exudates of Subarna (S) and Pankaj (P) using as solvent n-butanol:acetic acid:water (4:1:1), stained in 0.2% ninhydrin solution.

Similar bioassay experiments were also performed on IET 1444 seeds by Subarna RE; 1 mL RE of Subarna was used for preparative chromatography (Solvent: n-butanol:formic acid:water [5:2:5], upper phase). One vertical piece of the chromatogram was stained with $AgNO_3$ reagent to detect the spot.

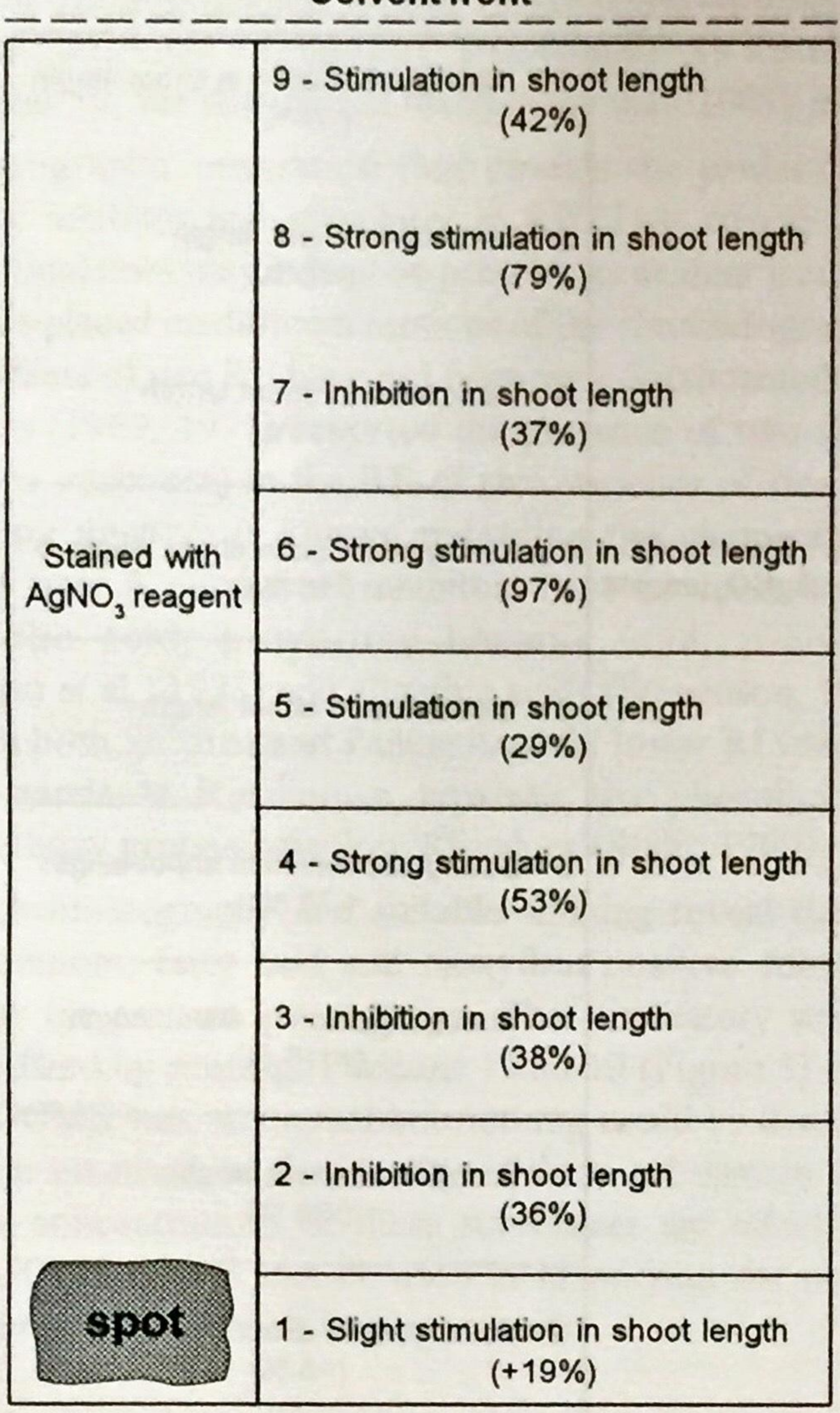

Figure 4. Stimulation and inhibition of Pankaj shoot length induced by RE of Subarna, fractionated by paper chromatography. (Solvent n-butanol:formic acid:water [5:2:5], upper phase).

The remaining larger unstained portion of the chromatogram was divided Inhibition was noticeable in section 1 (which corresponds to the stained spot) and in sections 4 and 7 (Figure 5). Two stimulators were also observed in

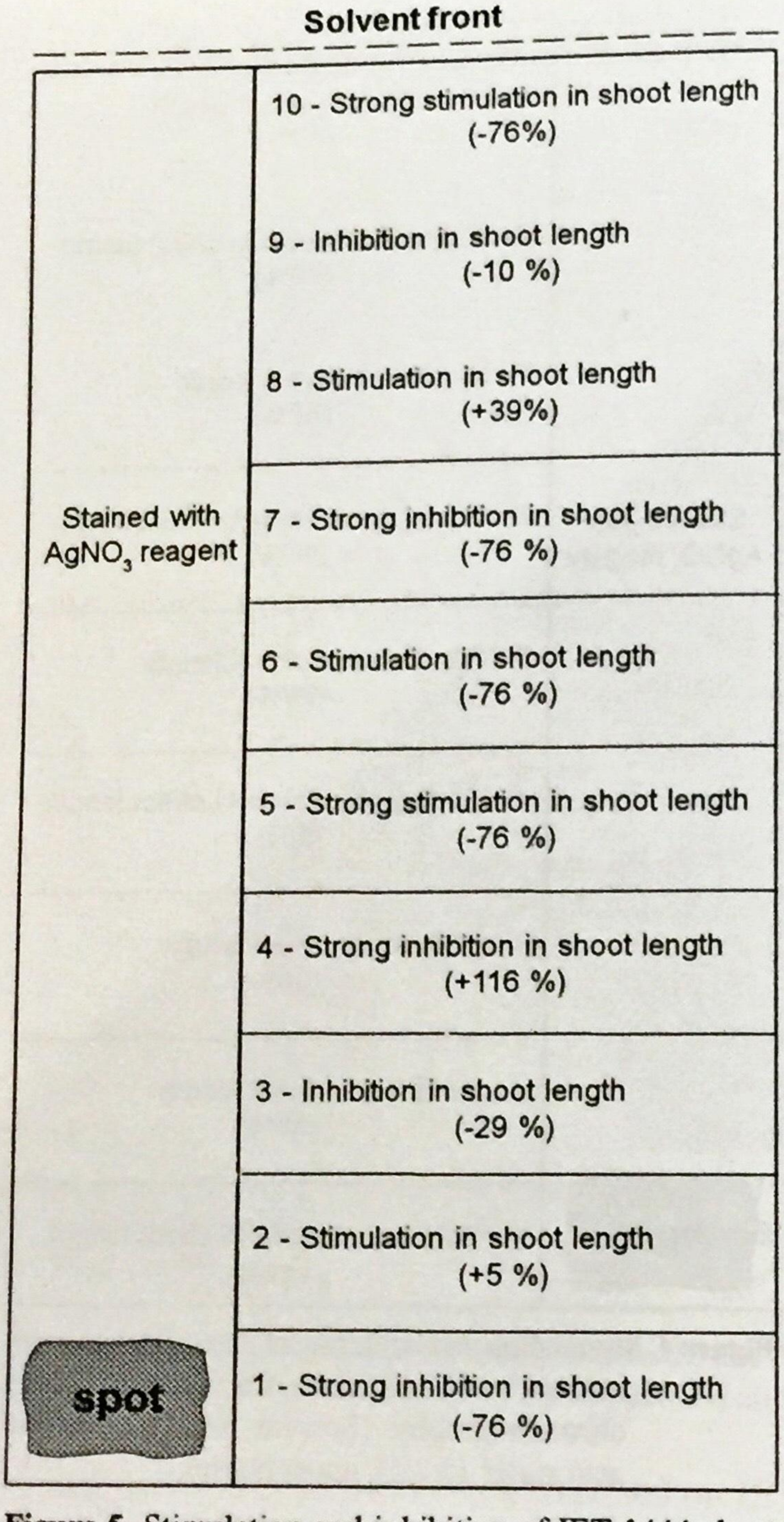

Figure 5. Stimulation and inhibition of IET 1444 shoot length induced by RE of Subarna, fractionated by paper chromatography (Solvent n-butanol:formic acid:water [5:2:5], upper phase).

Inhibition was noticeable in section 1 (which corresponds to the stained spot) and in sections 4 and 7 (Figure 5). Two stimulators were also observed in sections 5 and 10, the stimulation being more than 100% in these cases.

Chromatographic separation thus reveals the presence of multiple components of inhibitor and stimulator in RE. Two strong inhibitors and three strong stimulators may indeed be present, as evident from the bioassay on Pankaj seeds placed on different sections of the chromatogram of Subarna. Stimulatory effects of rice RE have not been well-documented (Rice, 1984). Sadhu and Das (1969, 1971) reported the presence of two stimulators (in addition to two inhibitors) in the RE of two varieties of rice (Rupsail and CB-1). Nothing positive is known regarding the chemical structure of stimulators of rice. A number of inhibitors have however been identified, namely vanillic acid, p-hydroxy benzoic acid, p-coumaric acid (Chandramohan et al, 1973) and alipathic acids (Stevenson, 1967). $AgNO_3$ spots in RE on both Subarna and Pankaj have far lower Rf values than those of the above standards. It is known, however, that phenolics with a larger number of methoxy groups have low Rf values (Stahl, 1969).

Paper chromatography and suitable staining reveal the presence of phenolic substances, fatty acid and ninhydrin positive substances in the varieties under the present investigation. The stimulatory substances have not been visualized by staining. Sections 1 and 10 (Figure 5) were eluted in acetone and GCMS was attempted but nothing could be detected. Nothing positive can be surmised on the identity of these substances. It thus seems that very low concentrations of these substances are effective inhibitors/ stimulators. GCMS or MS can be used in identifying the molecules after collecting the material in much larger amounts.

CONCLUSIONS

The results presented here reveal considerable allelopathic interactions between two varieties of rice, Subarna and Pankaj, apparently mediated through the RE. The over-all effect of RE is strong, unequivocal inhibition. As seen from the chromatogram, although the synergistic effect is inhibitory, stimulatory substances are also present in the RE of Subarna. As most of the allelopathic effects so far recorded in the rice plant are inhibitory, the

stimulatory substances are of special interest. Stimulators in Sections 4-6 (Figure 4) located in between inhibitors in Sections 3 and 7 are clear examples. So are those in 8 and 9 because they lie above inhibitor 7. Whether the wide variation in the stimulatory effects of 4-6 is due to more than one substance cannot be determined at present and the same is valid for 8 and 9. Finally, it can be concluded that at least two strong inhibitors and, likewise, two strong stimulators are present in the RE of Subarna.

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